Transport of Iodide and Other Anions in the Thyroid Gland

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I. INTRODUCTION

Iodine is the heaviest element normally metabolized in biological materials. Its weight probably also determines its scarcity in the earth's crust; iodine constitutes only an estimated $3 \cdot 10^{-6}$ % of the igneous rock (204), a somewhat greater fraction of soils, and $1-7 \cdot 10^{-6}$ % of sea water (155). The thyroid gland has made a most remarkable adaptation to this dearth of iodine by developing an extremely efficient mechanism for collecting this rare element. The process can be divided into two steps: iodide accumulation, and organic iodine formation and storage. This review is concerned with the first of these steps—iodide accumulation. The ability to concentrate iodide is the result of two processes: the entry process, which also called the one-way I^- clearance, "active" I^- transport, the I^- pump, or the I^- trap, and the exit process. The term I^--concentrating mechanism has been applied to the over-all process, and, unfortunately, so have the terms I^- pump or I^- trap.

A. Iodine-Concentrating Tissues

Tissues of vertebrates can be divided into two categories on the basis of their ability to concentrate iodide ion. The majority of tissues do not concentrate this anion above the level found in the plasma or surrounding medium. To this group, whose iodide level is one-half or less than that of plasma, belong such tissues as spleen, ovary, adrenal, lymph nodes, large bowel, liver, heart, skeletal muscle, lacrimal gland, pituitary gland, and brain (138, 141, 161, 176, 327). Even these low values are probably high because of contamination with blood and extracel-

lular fluid. (In some of the studies values listed are not specific for labeled iodide ion. When early time intervals are studied, before much organic iodine appears, this is not a serious error.) When extracellular fluid can be removed, as for example in ascites tumor cells, the tissue/medium ratio of iodide is reported to be low (265). Preliminary results suggest that the restriction of the intracellular I^- space of these cells to ~ 0.30 is the result of an active process (Salvatore and Wolff, unpublished observations). In *E. coli* the I^- space is about 10% less than the Na^+ space (264). In erythrocytes, iodide values are generally 10% higher than the chloride distribution (223). The best values are 0.515 for Cl^- and 0.565 for I^-; these become 0.73 and 0.80 when based on water content (33, 223, 235, 264). An upward shift is also seen as a result of the chloride shift when the red cells are exposed to CO_2. The extrathyroidal distribution space for iodide varies from 35 to 40% of the body weight in dog and man (152, 327) and over a somewhat wider range in the rat (45, 138). A considerable portion of this space may be accounted for by the gastrointestinal tract (¼ or more of the apparent I^- space is the result of concentration of I^- in saliva, stomach, and small bowel).

A surprising number of tissues fall into the second group, which exhibits iodide-concentrating ability. These are, in addition to the thyroid gland, gastric mucosa or its secretion, salivary glands or secretions, mammary gland and its secretion, choroid plexus, ciliary body, small intestine, placenta, ova, skin and hair, and the salt gland of the sea gull. In the ciliary body and choroid plexus, I^- (and SCN^-) is transported out of the cerebrospinal fluid (25, 26, 232a, 329). This accounts for the low ratio of cerebrospinal fluid/serum I^- (4a, 111a). The kidney shares certain properties of the iodide-concentrating tissues although the isolated organ does not concentrate iodide. Certain structures of lower animals, e.g., notochord and endostyle in the lamprey (180, 256), the byssus of the mussel (251), Balanoglossus (225), corals (252), and a variety of algae (65, 165, 168, 173, 257, 258, 276, 307, 337), also concentrate iodide.

As far as they have been tested, iodide-concentrating tissues of vertebrates exhibit remarkable similarities to thyroid tissue in their iodide-concentrating mechanism: *1*) inhibition by anions such as SCN^-, ClO_4^-, BF_4^-, and NO_3^-; *2*) concentration of a number of anions other than I^-; *3*) inhibition by various poisons such as 2,4-dinitrophenol and cardiac glycosides; *4*) a requirement for K^+; *5*) half-saturation with iodide near $\sim 3 \cdot 10^{-5}$ M I^-; and *6*) a possible genetic relation, as shown by the simultaneous loss of the ability to concentrate iodide from thyroid gland, salivary, and gastric secretions in two patients (25, 26, 46, 125, 294, 329, 347, 351). Certain differences have been pointed out (125). One is the failure of thyroid tissue to concentrate SCN^- to the extent found in saliva and gastric juice. This may well be only a quantitative difference. Another, the failure of extrathyroidal tissues to respond to thyrotropin, seems not to be serious since the thyroidal transport system is stimulated indirectly by the trophic hormone. Tissues enjoy their individuality partly because of a specific ability to respond to certain stimuli. This does not mean that the system stimulated may not be alike in most other respects from one tissue to the next. The same argument applies to high-iodine diets, etc., where, in the case of the thyroid, the metabolism of the ac-

cumulated iodide will produce secondary effects resulting from organic iodine formation. These would not occur in the other tissues where little organic iodine is formed. Much of the work on extrathyroidal iodide transport has been reviewed by Brown-Grant (46) and is referred to only for comparison with iodide transport in thyroid tissues. A summary is given in Table 1.

The notion that there exists in the thyroid gland a special mechanism for transporting iodide ion developed gradually in the 1940's. Leblond and Sūe (176, 177) found that after a large dose of labeled iodide to guinea pigs most of the thyroidal radioactivity was in the form of the anion. This was also shown at early intervals after the administration of smaller doses to rabbits (179). However, the concept that iodide transport can exist independent of hormone synthesis was first clearly stated by Schachner et al. (269) and Franklin et al. (87) in 1944. Using antithyroid compounds (sulfonamides and thiouracil) they showed marked inhibition of hormone synthesis without changes in the iodide-concentrating capacity of thyroid slices. They concluded that "there exists a mechanism for concentrating iodide that does not depend upon the conversion of inorganic iodide to thyroxine and diiodotyrosine." These findings were soon confirmed in intact animals (12, 316). ^{131}I concentration in thiouracil-blocked thyroid glands

TABLE 1. *Extrathyroidal iodide-concentrating tissues of chordates*

Tissue	Species, etc.	Comments	Ref.*
Salivary glands	Man, dog, guinea pig (parotid), man, mouse, hamster?, guinea pig (submaxillary), cat, rabbit (sublingual) *not* in rat	Concentrated by duct cells	46
Gastric mucosa (or juice)	All vertebrates investigated	Concentrated by mucous cells; independent of HCl secretion	46
Small intestine	Rat		46
Mammary gland	All species investigated	Some organic iodine in certain species; sizable I loss from body	46
Ovary or ova	Hen, amphibia, teleosts, cyclostomes		46, 181
Placenta	Rabbit, guinea pig, ? rat	Hemoendothelial placentas	46
Notochord	Cyclostomes	$I^- > Br^- > Cl^-$	181
Epidermis and hair	Rat, *Rana*		46, 181
Gills	*Scyllium*		181
Serum	Migratory teleosts: *Salmo*, *Alosa*, *Mugil*	Bound to "albumin"	181
Ciliary body-iris	Rabbit	Anion behavior like that in thyroid	25, 120
Choroid plexus	Rabbit		26, 329
Nasal gland	Herring gull	$Cl^- > I^-$	52

*More than 100 pertinent references can be found in each of the two most important reviews on this subject (46, 181).

was invariably greater than that of the plasma. This concentration factor, termed thyroid/serum ratio (T/S)[1] in experiments in vivo and thyroid or tissue/medium ratio (T/M) in tissue slice experiments, has been the basic yardstick for measurement in many of the experiments to date. It is a complicated function representing, as a minimum, the balance between influx and efflux of iodide, and therefore carries with it the limitations of such an over-all analysis. In some cases, notably in the work of Wollman, influx and efflux have been separated. However, much information has been gained from manipulations of the T/S[I⁻] or T/M[I⁻], and much of this review concerns itself with such data. Representative values of this ratio are 20 to 30 in the rat on a high iodine intake, ~75 in the guinea pig, 80 to 140 in the mouse, and 10 to 40 in sheep or beef thyroid slices (137, 218, 337, 360). When the gland is stimulated by moderate- or low-iodine diets, or TSH, these ratios may attain values greater than 100 to 200 in rats (340, 360), 400 to 500 in guinea pigs (345), 300 to 400 in chicks (Wolff, unpublished), and 200 to 300 in mice (360).

B. Nature of the Concentrated Iodine

Proof for the inorganic nature of the ^{131}I transported has been presented: *1*) After $^{131}I^-$ injection into animals treated with an antithyroid compound the thyroid radioactivity is nearly quantitatively soluble in the presence of protein precipitants (304, 352, 364); and *2*) it is readily dialyzable or ultrafiltrable (130, 213, 304, 313). Neither *1* nor *2* proves the radioactivity to be the anion, however. *3*) The material becomes quantitatively extractable into CCl_4 after treatment with acid iodate—subsequent reduction makes it again water soluble (87, 269, 304). This strongly suggests either I^- or I_2. *4*) Potentiometric titration (AgI electrode) is consistent with iodide (313). *5*) The electrophoretic behavior is consistent with I^- (213, 259). *6*) Last, the accumulated radioactivity in blocked thyroids of various species behaves chromatographically like I^- in a variety of solvents (130, 305, 306, 345, 356, 365, 368, 371). To what extent the activity of I^- is reduced by association with proteins of the thyroid gland is not known at present but should be kept in mind because of the high protein concentrations in the follicular lumen.

The finding (305) that the T/S[I⁻] determined by radioactivity was markedly less than that obtained from chemical measurement, and the older reports that thyroids contain sizable quantities of iodide (42, 119), led to the suspicion that there may be in the thyroid gland a second pool of iodide not in rapid equilibrium with the iodide entering the gland from the circulation (29, 130, 149, 248, 305,

[1] Abbreviations used are: T/S or T/M is the ratio

$$\frac{\text{concn. of substance/unit wet wt. } (\mu g \text{ or } g) \text{ of tissue}}{\text{concn. of substance/unit vol. } (\mu l \text{ or } ml) \text{ of serum or medium}}$$

S applies to serum (generally used for in vivo experiments). M applies to incubation medium of in vitro experiments. Volumes are chosen to give units of weight roughly equal to tissue weight units, i.e. mg and μl, but generally no corrections are made for density of serum or for water concentration of tissue or serum. The species concentrated is indicated when required, thus T/S[I⁻] is thyroid/serum ratio of iodide, T/M[ReO_4^-] the salivary slice/medium ratio of perrhenate, etc. TSH stands for thyrotropin.

355). This discrepancy was considered a methodological artifact (306, 367), but with the demonstration of intrathyroidal deiodination of mono- and diiodotyrosine (e.g. 253, 292), with the suggestion that in many cases a significant fraction of ^{131}I released from the thyroid was iodide presumably derived from organic iodine (211), and with the further suggestion (248) that antithyroid drugs may interfere with the internal recycling of I^- derived from deiodination, this question has been re-examined. It seems probable that I^- can enter the thyroid from several sources. Some of these sources are not labeled when organic iodine formation is blocked but become progressively labeled if organic iodine formation is permitted (130, 357, 364). This potential "pool" has been claimed to be not in equilibrium with the "pool" measured in the blocked gland because it is not discharged by ClO_4^- (130). However, under different experimental conditions, e.g. TSH stimulation and longer time intervals, ClO_4^- can apparently discharge I^- in excess of that in the "exchangeable" pool (215, 236, 260). Such continued I^- discharge over considerable periods of time can only be accounted for by release from organic iodine. Some part of the organic iodine may be a better source for I^- than the remainder, as judged from time courses of labeling of I^- in the "second pool" compared to the total for the gland (130, 229a). Whether the internal sources feed into a separate pool, what the amount of this contribution is, and what fraction of such I^- results from experimental manipulation cannot be decided at present because adequate methods for unambiguous measurement of very small proportions of total thyroid iodine have not been devised (e.g. 213). Since little is yet known of this "second pool" it has, of necessity, been largely neglected in most of the studies reported here.

II. METHODS

A. Organic Iodine Formation Prevented

The ability of thyroid tissue to concentrate the iodide ion can be studied in the untreated gland in which the anion is further metabolized, or under conditions in which "all" further metabolism of iodine is prevented. The latter system confines studies to the fate of iodide only, gives larger T/S or T/M ratios, is technically simpler, and has been used far more frequently.

1. Thiocarbamides. It was evident from the early studies that the thyroid's capacity to accumulate iodide ion could best be studied when as many of the competing reactions (i.e. hormone biosynthesis) as possible were blocked. To this end antithyroid agents or excess iodide have generally been used. The techniques are variations of the method devised by VanderLaan et al. (313, 318). Animals are injected with a dose of antithyroid agent that "completely" blocks organic iodine formation (e.g. 5–10 mg of propylthiouracil/100 g in rats) 30 to 60 minutes before $^{131}I^-$ is injected. The animals are usually killed 60 to 90 minutes later, but for each experimental condition it is essential to know at what time equilibration between thyroid and serum iodide occurs. Effective inhibition of organic iodine formation occurs rapidly—about 1 minute after intravenous injection in mice (359). [Complete inhibition of monoiodotyrosine formation is not obtained even with large doses (154, 229, 230, 364), but the error introduced is negligible for most experi-

ments. Large doses of many goitrogens appear to be toxic.] When small doses of propylthiouracil are given, T/S can be shown to be a linear function of the logarithm of the dose until "complete" inhibition of organification has occurred (368). Most studies are made on the assumption that the only effect of these agents is to block organic iodine formation, but this may not always be the case (359). The choice of antithyroid agent is of some importance. While a number of thiocarbamides and sulfadiazine give essentially similar results, the T/S[I^-] with 2-mercaptoimidazole is higher (in mice) while several other agents give low T/S values (368).

In vivo counting over the neck has been used to measure saturation or discharge of concentrated I^-. This is qualitatively a very useful technique, especially to show failure to form organic iodine, but is quantitatively unreliable. The discharge of I^- tends to be incomplete (352), and there is a simultaneous increase in the neck background.

2. Excess iodide. One of the astonishing properties of the iodide-concentrating mechanism is its ability to accumulate enough iodide ion to inhibit the further metabolism of iodine (207, 234, 339, 340). Both the iodination of tyrosine and the subsequent coupling reaction to form thyroxin are inhibited (341), and the proportion of ^{131}I present as monoiodotyrosine is elevated during the period of minimal iodide binding (97). Inhibition persists as long as the plasma iodide level remains above 20 to 35 μg/100 ml (thyroid iodide level >2 mg/100 g) (339), that is, at levels well below saturation of the I^--concentrating mechanism (see Table 2). However, after 16 to 40 hours the gland becomes refractory to further inhibition (97, 342), apparently because the ability to concentrate enough I^- is lost (42a, 359). Carefully chosen doses of SCN^- can reduce intrathyroidal I^- sufficiently to allow the synthesis of organic iodine. Contrariwise, stimulation of the I^--trapping mechanism by TSH can elevate the T/S[I^-] sufficiently to lead to inhibitory iodide concentrations in the gland at lower plasma I^- levels (234). Some 50 cases of iodide-induced goiter and/or myxedema have been reported in the clinical literature. Whether this implies a failure to escape from iodide inhibition, or a special sensitivity, remains to be determined.

As with other antithyroid agents, organic iodine synthesis is not completely inhibited by excess iodide, but the error is not serious if the proper experimental design is chosen. The use of such large doses of carrier eliminates various experi-

TABLE 2. *Comparison of K_m values for iodide in mammalian tissues*

Tissue	Measurement	K_m, M	Ref.
Mouse thyroid	in vivo	$2.6 \cdot 10^{-6}$	356, 365
Sheep thyroid	in vitro	$3.0 \cdot 10^{-5}$	347
Rat thyroid	in vivo	$2.5\text{–}8 \cdot 10^{-5}$	122, 137, 365
Mouse submaxillary	in vitro	$4 \cdot 10^{-6}$	347
		$\sim 1 \cdot 10^{-4}$	85
Rat mammary	in vitro	$4 \cdot 10^{-4}$	347
Rabbit choroid plexus	in vitro	$5 \cdot 10^{-6}$	26
Rabbit ciliary body—iris preparation	in vitro	$2 \cdot 10^{-6}$	25

ments from consideration. For most purposes, therefore, iodide is a less suitable antithyroid agent than the thiocarbamides.

3. Abnormal thyroid tissue. Clinical material has provided an opportunity for studying the isolated iodide-concentrating mechanism in human thyroid tissue. Certain familial goitrous cretins (see review 293), as well as patients with Pendred's syndrome (88), have a limited or virtually nonexistent capacity to oxidize iodide ion. In these cases iodide accumulates as in the goitrogen-blocked thyroid, and certain comparable studies are possible. As far as studied, the behavior of these pathological glands resembles that of experimental animals given thiocarbamides.

A transplantable rat thyroid tumor, developed by Wollman (361), is qualitatively like the blocked thyroid gland (347, 352, 361), although the fluxes appear to be less. Nevertheless, because of the bulk of tissue available (grams), this tumor makes a useful tool for studying the concentrating process without drugs.

4. Importance of equilibration. Implicit in the time schedule usually used is the important assumption that a steady state between influx and efflux of iodide has been attained (111, 127, 130, 138, 337, 347, 357, 360, 361, 364, 365). The importance of making comparisons only when equilibration has been attained was not realized in early studies (134, 313, 316, 318). The 60- to 90-minute period usually employed in rat studies is probably safe, but certain other studies must be interpreted with caution (281–284). In rats, the steady state is attained in 30 to 40 minutes, whereas in mice the time is 40 to 60 minutes (360, 364) (see Table 3). Once equilibration has been attained the T/S remains constant over a considerable period of time despite a falling blood $^{131}I^-$ level as the anion is cleared by the kidney (46, 304, 347, 364).

For the purposes of this discussion we assumed that measurement of $^{131}I^-$ reflects behavior of $^{127}I^-$. This may not always be strictly correct, e.g., if equilibrium is not attained, if blood $^{131}I^-$ falls very rapidly (355, 368), or in slice experiments where the specific activity will be diluted by iodide leached out of the slice (271) (see p. 52).

5. Heterogeneity of iodide. The thyroid iodide is measured as an average of several heterogeneous pools which complicate interpretation. Adjustments for several of the factors involved can be made in some cases and disregarded in others, but should at least be kept in mind.

a) From the T/S or T/M should be subtracted that concentration of anion that can be considered as entering by "diffusion." This quantity is more accurately defined as the T/S or T/M at infinite anion concentration or the T/S in the absence of active transport. For iodide it has been termed C or D (see below) and amounts to about 0.4 of the outside iodide concentration in the mouse or rat. Values for C or D have been determined: at very high iodide levels (122), when transport was blocked by ClO_4^- (135, 138), by curve-fitting (111, 356), and in thyroid tumors lacking an iodide-concentrating mechanism (369). The nature of the entry process represented by C or D is probably complex, and the reason for the restriction of this space to 0.4 of the external iodide concentration is not clear.

b) Although different follicles vary less with regard to the accumulation of I^- compared to organic iodine (71, 211, 231), heterogeneity between follicles exists

TABLE 3. *Kinetic constants of iodide transport*

Diet Etc.	T/S[I⁻]	One-Way Clearance, μl mg⁻¹ min⁻¹	K_{TS}, min⁻¹	Extraction Ratio	Binding Rate Constant K_b	Time Est. for 95% Equilibration, min		Ref.
						Blocked	Unblocked	
Mouse								
High I	86	4.7	0.050		0.15	60	20	360
Mod. low I	220	9.0	0.041		0.86	73	3.5	360
Hypophysect., mod. low I	76	2.4	0.025					360
Rat								
High I	29	3.2	0.11		0.32	27	9.3	360
Mod. low I	125	15.0	0.12		1.70	25	1.8	360
Hypophysect., mod. low I	25	1.5	0.050					360
Hypophysect., high I		0.27	0.107					111
Rabbit								
?Mod. low I		1.1–12.2 2.9		0.25–0.57 >0.5				47, 206, 288
Dog								
?				0.3				260
Man								
Euthyroid		~0.8–2.5[1]		0.19, 0.20				30, 210, 232, 248, 295

and would also be expected in the degree of interference from any other sources of I^- (e.g. from deiodination).

c) No account is taken of the true water concentration either in thyroid tissue or medium. Such a correction would tend to increase the T/S as the water content of thyroid tissue is less than that of serum.

d) A certain degree of interaction with serum proteins (especially albumin) and iodide occurs (see p. 78). This tends to lower the concentration of free iodide. The importance of this has not been fully evaluated (356).

6. Thyroid slices in vitro. The usual advantages of in vitro techniques apply here, and best results are obtained when slices from a single gland are used. Incubation media containing an antithyroid agent (10^{-2}–10^{-4}M) are used. The nature of the agent seems to be less critical here than in intact mice. Equilibration usually occurs between 50 and 100 minutes, depending to some extent on the final T/M[I⁻] attained (337, 347, 348). The value for C or D is greater in vitro and amounts to ~0.75 (347). Part of this greater size may be contributed by adhering medium, part by an increased extracellular space [approx. 0.3 in normal rat thyroid in vivo (375) and 0.4–0.6 in slices (Debons and Pittman, personal communication)]. A potential error stems from inhibition by materials released from the slice into the medium (205). This can be reduced by using small amounts of tissue compared to

the medium or by flushing the tissue with a continuous change of fresh medium (205, 337, 347). The various iodinated amino acids do not appear to be inhibitory agents (124, 136, 305, 352), but iodinated peptides may be responsible (187). Iodide released into the medium amounts to 0.5 to 1.0 μg I^-/g thyroid in the above experimental conditions (271). With small amounts of tissue (compared to volume of medium) this has a negligible effect on saturation.

B. T/S[I^-] in Unblocked Glands

It may well be argued that measurement of T/S[I^-] when organic iodine formation is blocked introduces artificial conditions that may not reveal the normal course of events. Several investigators have studied the T/S[I^-] ratio in the presence of active hormone biosynthesis (306, 362, 366). While study of the unblocked thyroid is important, this system has been less often used because it has several disadvantages.

a) Foremost, there is an unknown amount of decomposition of organic ^{131}I.

b) The ratios are often lower and hence measurements less accurate. This decrease results from the fact that the intrathyroidal iodide pool is constantly being depleted by the process of organic iodine formation. The "simple" influx-efflux steady-state level of I^- established in the blocked gland is thus never attained.

c) Since organic iodine is continually being hydrolyzed and then deiodinated (e.g. 253, 292), radioiodide enters the thyroid gland from a source other than the surrounding medium and hence must be separately considered. Whether or not this iodide reaches the same "areas of concentrated iodide" as does the exchangeable portion is still a matter of some uncertainty (130, 357, 364, 367). In addition, this method requires separation of organic and inorganic iodine, which would tend to increase these problems. They can be minimized by the use of early intervals (130, 305, 306, 364).

d) Last, various experimental procedures may act on the organification process rather than on the concentrating mechanism (e.g. 358) and thus give differences in T/S[I^-]. Nevertheless, important kinetic conclusions have been reached in this system, especially regarding the relative rates of the trapping and organification processes.

Anion accumulation in the absence of drugs can also be measured by the use of nonmetabolized anions such as ReO_4^- or TcO_4^-. They could, furthermore, be used in conjunction with I^-, whose metabolism could be studied simultaneously (10).

The T/M[I^-] can also be studied in the unblocked thyroid slice. In this in vitro system, however, a larger proportion of the total accumulated radioactivity will be in the form of iodide than in the thyroid gland in situ. Most of the information so far collected has been obtained in blocked thyroid glands, and much of the information presented below comes from such experiments.

III. ANATOMIC CONSIDERATIONS

Radioautographic studies with thyroid glands in which organic iodine formation was blocked by thiouracil suggested a very rapid transfer of $^{131}I^-$ into the follicular lumen (freeze-drying techniques are necessary in such studies to avoid

loss of $^{131}I^-$ into the fixative). An earlier, cellular stage for $^{131}I^-$ has not yet been reported. Much more radioactivity appears in the lumen than in the cells (71, 231). [It is of interest that in the Graafian follicle a period of cellular concentration of $^{131}I^-$ can be demonstrated before radioactivity finally collects in the follicular lumen (27). The very slowness, however, may limit the utility of this comparison.]

Cellular integrity is required for demonstration of iodide transport. When thyroid tissue is homogenized, placed into a dialysis bag, and suspended in a medium containing $^{131}I^-$, iodide ion is not concentrated significantly. Even high concentrations of homogenate (50 %) fail to concentrate iodide, provided suitable precautions are taken to prevent formation of organic iodine (72, 348, 349, 377). Small gradients may be obscured by contributions of the Gibbs-Donnan equilibrium, but would not be likely at the pH that usually obtains. On the whole, this evidence [as well as energy requirement, high temperature dependence, and effect of certain inhibitors (see below)] rules out simple adsorption as the main mechanism for maintenance of iodide concentration. Even freezing of thyroid tissue abolishes the ability to concentrate iodide though the ability to form monoiodotyrosine is retained (200). On the other hand, it takes greater acute radiation damage to impair I^--concentrating ability than a number of other metabolic processes of the thyroid (19a).

In an attempt to find out whether or not the follicle as such is necessary for the iodide transporting process, considerations of studies on fetal thyroid glands are helpful. Inorganic and organic iodine (^{127}I) have been detected in fetal calf thyroids by Wolff and co-workers (170, 343) before differentiation into the follicular pattern. A number of studies have shown that ^{131}I can accumulate in the undifferentiated thyroid of the tadpole (192), chick (300, 310, 311, 371), sheep (19), and man (8, 57, 150). In most of these cases the presence of a specific mechanism for concentrating iodide was not demonstrated. Nevertheless, Wollman and Zwilling (371) proved that most of the ^{131}I of the "undifferentiated" thyroid of the 7-day chick embryo was not precipitable by trichloracetic acid and was in rapid equilibrium with the blood. This suggested iodide, and high ratios existed in such tissues. The ^{131}I was iodide by chromatography. Trunnell and Wade (311) showed a stepwise appearance of the various labeled iodinated compounds in order of complexity from 7½ to 9¾ days of embryonic life. Iodide preceded the appearance of all other compounds, as it did in the rabbit fetus (328a). It thus seemed likely that the follicular lumen was not necessary for iodide concentration.

This issue has finally been settled by studies with isolated thyroid cells prepared by trypsin treatment. Tong et al. (308) prepared isolated thyroid cells capable of accumulating iodide against a concentration gradient. When organic iodine formation in such cells was blocked, the cell/medium ratios for iodide ion attained a value of 6 to 9. Because of the techniques used this is a minimum value. The accumulated iodide was discharged by ClO_4^- (another criterion that ^{131}I was present as the anion). The results of such studies suggest strongly that the follicular pattern of thyroid architecture is not necessary for the ability to concentrate iodide ion. This is a property of the thyroid cell.

It seems probable from radioautographic studies (71, 231) and high T/M[I^-]

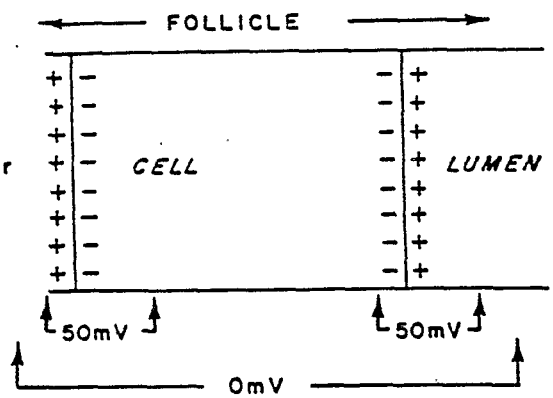


FIG. 1. Electrical potential across the basal and apical membranes of the thyroid cell according to Woodbury and Woodbury (375). There is a potential difference of ~50 mv into the cell from both capillary and luminal sides so that the net potential drop from capillary to lumen is ~0 mv.

ratios often attained by intact follicles but not by isolated cells, that the follicular lumen, or at least the organization into follicles, is important. Woodbury and Woodbury (375) have measured the voltage drop across the basal and apical membranes of the thyroid cell. They proposed the scheme shown in Fig. 1. A possible interpretation is that once I^- is concentrated against the electrical potential of the basal membrane, it may then "flow downhill" *with* the electrical gradient across the apical membrane into the lumen. Others (182a) find higher potential differences across the basal membrane while the two sides of the apical membrane are isopotential. Both these findings point to the basal membrane as at least one site for I^- concentration. The importance of the lumen as a storage region is nevertheless clear from radioautographic studies (71, 231). If there is no net voltage drop between blood and lumen, the cell could be considered as a barrier between two extracellular compartments. In the direction of exit a similar electrical barrier would exist but here iodide moves with the chemical gradient. The fraction of the total volume of the thyroid occupied by cells appears not to be directly related to the ability to concentrate iodide (130, 138, 318).

IV. KINETICS

A. Criteria for Active Transport

Active transport is often defined by exclusion, i.e., it is what remains after other forms of transport have been explained. Nevertheless, it is generally considered to be an *uphill transport against a gradient*—an electrochemical gradient in the case of charged substances, and a chemical gradient in the case of neutral substances. Not all workers studying transport concur in this definition. Transport, not necessarily uphill, but requiring participation of cellular metabolic activity, has also been considered "active." In any event, the process requires energy supplied by another system.

In the case of I^- transport into the thyroid gland, the more rigorous of the above criteria would appear to have been fulfilled. So far we have shown *1*) that I^- ion is concentrated against both a chemical and an electrical gradient; *2*) that the anion appears not to be bound to any extent; and 3) that cellular integrity is required for this process, i.e., simple adsorption cannot explain the observed iodide concentration. Additional evidence on the nature of the concentrating process is presented in later sections. Briefly, this is: *4*) the system is saturable with iodide; *5*) related anions inhibit iodide concentration competitively; *6*) oxidative metabolism and phosphorylation are required; *7*) there is a high temperature coefficient. In sum, these criteria satisfy the conditions for active transport of iodide. This applies to the over-all process and does not state at which membrane this process occurs. While there are many steps involved, a convenient description of the transport rate as the present state of knowledge deals with *a*) kinetic and *b*) energetic parameters. In the present section, we deal with kinetic considerations. While the effect of inhibitory anions properly belongs here as well, the wealth of material has led us to defer discussion of them to a separate section (VI).

B. Models

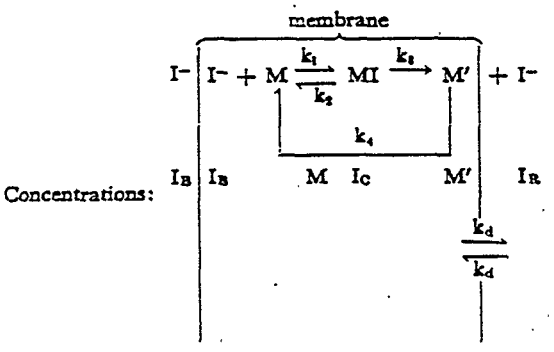
Present knowledge of iodide transport is so inadequate that reasonable models have to omit a number of factors that one is tempted to include. Thus the "electrical" component is deleted from kinetic treatments, transport is considered as occurring across a single membrane only, etc. Moreover, many of the rate constants of present models cannot be evaluated explicitly because of technical limitations; e.g. we are unable to determine the cellular and luminal iodide compartments separately. Nevertheless, a number of models have been devised that are in good agreement with experimental data and yield useful empirical constants.

1. Steady-state models. Wollman and Scow (365) have shown that a number of steady-state models can be constructed to give the same general equation

$$T/S[I^-] = \frac{I_T}{I_B} = \frac{A}{K_m + I_B} + C \qquad (1)$$

where I_T and I_B are thyroid and blood I^- concentrations, respectively, and A, C, and K_m are constants whose interpretation depends on the model.

The simplest model depends on adsorption alone and is, almost certainly, not applicable to the thyroid. In the simplest "active" transport model I^- entry occurs by an "active" or saturable process and by "diffusion." In the "active" process iodide combines reversibly with a carrier in the membrane and is then unloaded irreversibly into an area of high I^- concentration. Since the concentrated I^- appears to be chemically unchanged, the irreversibility can be visualized most simply as resulting from some change in the carrier. However, a pore theory with fixed charges is in no way ruled out. The exit process is considered to be one of simple diffusion. A modification of previous models (365) which takes into account the effect of renewal of carrier has been devised by Dr. C. G. Lewallen (personal communication; 236):



where:

- I_B is the concentration of I^- that has diffused to site of interaction with carrier M (it is considered the same as serum I^-)
- I_R is the concentration of I^- in the region of concentrated I^-
- M is the concentration of free carrier
- I_C is the concentration of iodide-carrier complex = MI
- M′ is the concentration of altered carrier
- M_T is the total carrier concentration = $M + I_C + M'$
- $k_1 - k_4$ are rate constants
- k_d is the effective diffusion constant

A unit volume of thyroid is divided into the fraction in which concentrated I^- exists, F_R; a fraction in which diffused I^- exists, F_B; a fraction from which I^- is excluded, F_E, equivalent to 1-C on p. 52; and a fraction occupied by the membrane F_C. Note that F_C is included in F_B since I^- is assumed to diffuse into the membrane thus $F_R + F_B + F_E = 1$. The total thyroid I^- concentration then becomes

$$F_R I_R + F_B I_B + F_C I_C = I_T \qquad (2)$$

I_R can be solved in terms of M_T from the differential rate equations for the reactants set equal to 0 for the steady state:

$$I_R = \left[\frac{k_3 k_4}{k_d(k_3 + k_4)} F_C M_T I_B\right] \Big/ \left[\frac{k_1(k_2 + k_3)}{k_1(k_3 + k_4)} + I_B\right] + I_B$$

Substituting into *equation 2* and transposing I_B:

$$T/S[I^-] = \frac{I_T}{I_B} = \left[\frac{k_3 k_4}{k_d(k_3 + k_4)} F_C M_T\left(F_R + \frac{k_d}{k_3}\right)\right] \Big/ \left[\frac{k_4 (k_2 + k_3)}{k_1 (k_3 + k_4)} + I_B\right] + F_R + F_B \qquad (2a)$$

Note that $F_R + F_B = C$ of Wollman's notation. Setting the numerator equal to A and letting $K_m = [k_4(k_2 + k_3)]/[k_1(k_3 + k_4)]$ the equation reduces to *equation 1*.

The general case for competitive anions must account for the fact that most of these (except perhaps for SCN^-) are concentrated also, i.e. k_3' is not zero. These anions are then more akin to competitive substrates than to the classical competitive inhibitor. Thus the corresponding equation for $T/S[I^-]$ in the presence of the competitive ion, G (concn. in medium = G_B), whose rate constants in the model are k_1', k_2', k_3' (k_4 remains the same), will be (236):

$$\frac{I_T}{I_B} = \left[\frac{k_3 k_4}{k_4(k_3 + k_4)} F_C M_T\left(F_B + \frac{k_4}{k_3}\right)\right] \Big/ \left[\frac{k_1(k_3 + k_2)}{k_1(k_3 + k_4)} + \frac{k_1'(k_3' + k_4)(k_3 + k_2)}{k_1(k_3 + k_4)(k_3' + k_4')} G_B + I_B\right] + F_B + F_S \quad (3a)$$

By multiplying the second term of the denominator by k_4/k_4 it can be seen that this term reduces to $(K_m/K_i)\,G_B$, where $K_i = [k_4(k_2' + k_3')]/[k_1'(k_3' + k_4)]$. It is an expression analogous to the K_m, in this case the K_m for the second anion. The inhibitor equation reduces to:

$$T/S[I^-] = \frac{I_T}{I_B} = \frac{A}{K_m + \frac{K_m}{K_i} G_B + I_B} + C \quad (3)$$

—an equation analogous to that described by Wollman (356).

The empirical constants K_m and K_i are readily determined by graphical analysis of straight-line transforms of *equations 1* or *3*. Thus in

$$\frac{1}{T/S[I^-] - C} = \frac{K_m}{A} + \frac{1}{A} I_B \quad (1b)$$

a graph of $1/(T/S[I^-] - C)$ vs. I_B will yield a straight line with a slope of $1/A$ and an ordinate intercept of K_m/A (356). Wolff and Maurey (347) have used the transform

$$\frac{1}{I_T - CI_B} = \frac{K_m}{A} \cdot \frac{1}{I_B} + \frac{1}{A} \quad (1c)$$

In this case a plot of $1/(I_T - CI_B)$ vs. $(1/I_B)$ yields a straight line with slope K_m/A, intercepts $1/A$, on the ordinate and $-1/K_m$ on the abscissa. The K_m values of various tissues thus determined, or calculated from the literature, are listed in Table 2.[2] The K_m values also remain remarkably constant with different levels of TSH stimulation (365).

The K_i can be obtained graphically as the common intercept of a family of lines corresponding to different fixed values of I_B in the coordinate system $1/(I_T - CI_B)$ vs. inhibitor concentration (92, 349). The assumptions involved have

[2] The test of an empirical equation is whether it fits the data. It appears to do so in the cases so far tested (347–349, 356, 365) except for two points in a single experiment (125). A twofold range may be expected as the experimental error. It is apparent that interpretations of the constants A, K_m, and K_i are entirely dependent on the model used. K_m is an equilibrium constant (k_2/k_1) only in the simple adsorption model (44). K_i is considered an equilibrium constant in simple enzyme kinetics when $k_3 = 0$, but is not so considered in the present model. K_m would resemble the Michaelis constant $(k_2 + k_3)/k_1$ in a model in which carrier renewal, etc., was omitted from the expression. Similarly the $1/A$ term is not a simple analogue of $1/V_{max}$ of Michaelis-Menten notation. While it certainly contains a capacity term, the complexity of A in the active transport model does not make the $1/A$ intercept useful for determination of the number of "sites," etc. Several authors have determined the capacity of the iodide-concentrating mechanism of the thyroid for iodide experimentally. The "normal" rat thyroid is "saturated" by 10–20 mg I^-/100 g fresh tissue, the guinea pig at slightly higher values (122, 177, 313). This is reduced considerably by hypophysectomy and increased to 40 mg I^-/100 g by TSH and to 80 mg I^-/100 g by chronic propylthiouracil treatment. From what has been said it is not surprising that TSH produces an increase in the value of A (122, 365).

been discussed by Reiner (243). It is of interest that the K_m for ReO_4^- is the same as a K_i for this anion measured against I^- (349).

2. Compartment analysis of equilibration curves. If the charge of the iodide ion is ignored and the animal is assumed to be in iodine balance, several elegant and informative analyses can be made from equilibration curves of blood iodide with thyroid iodine (29, 360, 364, 366). The simplest model possible has been used when consistent with observed data. But, as will be seen, a number of conditions may arise requiring introduction of additional compartments for the best fit of the data.

A. TWO-COMPARTMENT MODEL. The $T/S[I^-]$ describes not only the ratio of the concentrations, but also the ratio of the rate constants for influx and efflux. Using a simple two-compartment model Wollman and Reed (360) have derived the expression

$$T/S[I^-] = \frac{I_T}{I_B} = \frac{C/m}{K_{TB}} \tag{4}$$

where I_T and I_B are thyroid and serum iodide concentrations, respectively (the latter is assumed to be constant); C is the unidirectional iodide clearance, volume per minute; m is the mass of thyroid tissue: and K_{TB} is the exit rate constant (in min^{-1}).

The fundamental equation for transport in the blocked gland is:

$$(dI_T)/dt = C/m(I_B) - K_{TB}(I_T) \tag{5}$$

At early time intervals after iodide administration I_T can be neglected, and *equation 5* reduces to

$$(dI_T)/dt = C/m(I_B)$$

Thus

$$C/m = \frac{(dI_T)/dt}{I_B} \tag{6}$$

This method works as long as K_{TB} is not too large, and the reaction is far from equilibrium.

A crude estimate of K_{TB} can now be obtained from *equation 4*. This value can then be adjusted to give a constant value of C/m in the expression:

$$C/m = \frac{(dI_T/dt) + K_{TB}\, I_T}{I_B}$$

The final estimate is then made from a graphical integration of the integrated form of *equation 5*

$$I_T = \frac{C}{m} e^{-K\, TB^t} \int_0^t I_B(t)\, e^{K\, TB^t}\, dt \tag{7}$$

and adjusting K_{TB} to give a curve that best fits the experimental points. The shape of the equilibration curve is explicitly determined by K_{TB} and I_B.

Iodide clearances calculated in this manner or by several others are listed in Table 3. It is apparent that there can be great variation with the state of thyroid

activity as influenced by disease states, species, TSH stimulation, or diet. Even the less stimulated thyroids show clearances that imply a very sizable blood flow. Because of the complicated vascular arrangement of the thyroid (e.g. 51, 203), and changes of vascular tone and/or blood flow resulting from anesthesia and ligation of vessels, measurements of blood flow can be quite inaccurate (e.g. 51, 288). Crude estimates of the extraction ratio (i.e. arterial ^{131}I-venous ^{131}I/arterial ^{131}I) vary from 0.15 to 0.75 (206, 232, 248, 260, 288, 289). The calculated volumes of flow are 3 to 7 vol blood/vol of gland/min. Estimates made from a blood iodide level of 3 μg/liter (man) and a daily iodine requirement of 70 μg/day yield similar values (248, 289). Much higher values will be encountered in active glands, and in thyrotoxic glands clearances >1000 cc/gland/min have been measured, which makes such clinical phenomena as a thrill or bruit very understandable indeed (30, 210).

B. THREE-COMPARTMENT MODEL. The simple open two-compartment model describes many of the events usually measured; nevertheless radioautographic evidence (see section III) requires use of a more complicated model, one in which two thyroidal iodide compartments are to be accounted for. Therefore, Wollman (360) has expanded his simple model into an open three-compartment catenary model which considers iodide transport across both basal and apical membranes of the thyroid cell, i.e., one in which there is a cellular and a luminal iodide compartment (see Fig. 2), and one which demonstrates the ambiguity of K_{TB}. The differential equations for iodide uptake by this model are:

$$\frac{dI_c}{dt} = \frac{C}{V_c} I_B - K_{CB} I_C - K_{CL} I_C + K_{LC} \frac{V_L}{V_C} I_C \qquad (9)$$

$$\frac{dI_L}{dt} = K_{CL} \frac{V_C}{V_L} I_C - K_{LC} I_L \qquad (10)$$

(Symbols are defined in legend of Fig. 2)

Since radioautographs show most of the radioiodide in the lumen (71, 231), it may be assumed that

$$K_{CL} \gg K_{LC} \text{ and } K_{CB}$$

The over-all exit rate constant K_{TB} now becomes

$$K_{TB} = \frac{K_{LC} K_{CB}}{K_{CL}}$$

This can be increased by inhibiting cell-to-lumen transport of iodide without a change in clearance, whereas in the two-compartment model such inhibition would affect the one-way clearance.

While the determination of the individual rate constants is technically impossible at present, this three-compartment model allows a reasonable view for the competitive nature of inhibitors that are not themselves concentrated and takes into account the anatomical evidence.

3. Kinetics in the unblocked thyroid. If organic iodine formation is permitted, the simplest description is for a three-compartment model (362), i.e., in which the

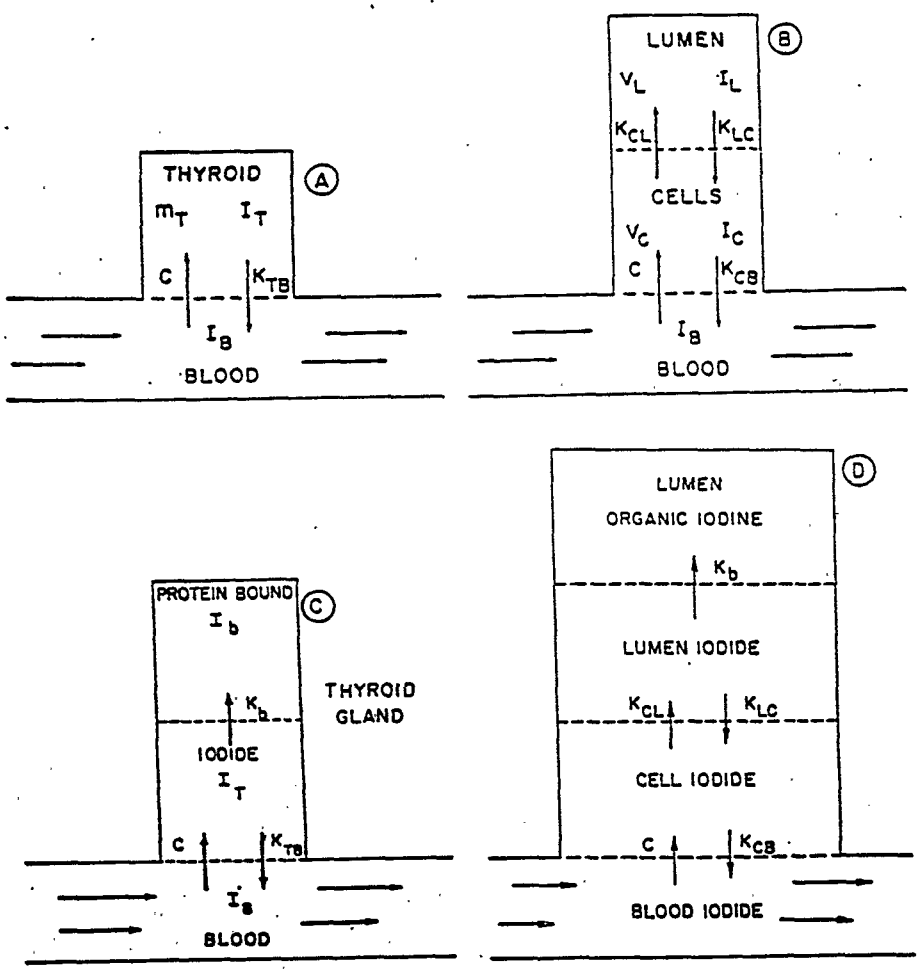


FIG. 2. Various kinetic models for iodide transport proposed by Wollman (358, 360, 362). *A:* simple two-compartment model with organification blocked by an antithyroid agent. *B:* three-compartment model with organification blocked. The thyroid compartment is now divided into cell and lumen with separate transport steps across basal and apical membranes. *C:* three-compartment model with organification allowed. The thyroid compartment is divided into an organic (protein-bound) and inorganic (iodide) compartment without consideration of the location of the processes. *D:* Four-compartment model that combines organification and anatomical considerations of models *B* and *C*. The symbols are:

I_B, I_T, I_C, or I_L are blood, total thyroid, cell or lumen iodide concentrations;
I_b organic I^{131} concentration of thyroid;
C is iodide clearance (vol/min);
m_T is mass of thyroid;
K_{TB} is exit rate constant for iodide from thyroid considered as a whole (fraction/min);
K_{CL}, K_{LC}, K_{CB} are rate constants for iodide transfer from cell to lumen, lumen to cell, and cell to blood;
K_b is rate constant for organic binding of iodine;
V_C, V_L are volumes of cell or lumen.

organic iodine pool has been added. Since an unknown portion of the labeled organic iodine is deiodinated, early intervals are especially important here. Provided the reaction is first-order, the rate of formation of organic iodine is:

$$\frac{dI_b}{dt} = K_b I_T \qquad (11)$$

where I_b = organic iodine, K_b = the binding rate constant (for organic iodine formation).

The effective clearance is measured by both thyroid iodide, I_T, and the organic iodine formed, I_b, that is:

$$\frac{d(I_T + I_b)}{dt} = C/m\, I_B - K_{TB} I_T \qquad (12)$$

The time dependence of thyroid radioiodide concentration then becomes

$$\frac{dI_T}{dt} = \frac{C}{m} I_B - (K_{TB} + K_b) I_T \qquad (13)$$

which is integrated to

$$I_T = \frac{C}{m} e^{-(K_{TB}+K_b)t} \int_0^t I_B(t) e^{(K_{TB}+K_b)t}\, dt \qquad (14)$$

This equation is of the same form as *equation 5*. The steady-state equation for the $T/S[I^-]$ becomes

$$T/S[I^-] = \frac{C/m}{K_{TB} + K_b} \qquad (15)$$

when I_B is constant. Thus when organification is blocked $K_b = 0$ and the equation reduces to *equation 4*.

For a further refinement, which takes account of the fact that much of the organic iodine appears to be formed on the lumen side of the apical cell border (370), the reader is referred to the open four-compartment model described by Wollman (358).

While the processes across the single (basal or apical) membrane cannot be measured explicitly at present, it is clear from *equation 15* that the $T/S[I^-]$ will be smaller in the unblocked than in the blocked gland, depending on the size of K_b. This tends to be from 3 to 10 times greater than K_{TB} (362). At the same time it can be shown that equilibration will be faster in the unblocked gland. A summary of the pertinent data obtained by Wollman from compartment analysis is listed in Table 3.

V. REQUIREMENTS FOR ANION CONCENTRATION

Nearly all studies attempting to elucidate the requirements for energy, cofactors, etc., have been carried out in slices since they are the simplest readily available I^--transporting system.

A. Energy Requirements

1. Oxidative metabolism. The presence of oxygen is necessary for iodide transport but a specific substrate requirement for iodide transport has not been demonstrated. Thyroid slices perform about as well in the absence of added Krebs cycle intermediates or carbohydrates as in their presence. The nature of the endogenous substrate is unknown. Inhibitors of the metabolism of various substrates, e.g. arsenite or fluoroacetate, depress the T/M[I^-] at rather high concentrations (10^{-2} M). Malonate was ineffective (91). Anaerobiasis and inhibitors of oxidative processes such as CN^-, sulfide, azide, or *p*-phenylenediamine depress the T/M[I^-] more or less in parallel with the O_2 consumption (87, 90, 91, 269, 287). Azide may be a special case in that it also is an anion belonging to the pseudohalogens.

2. ATP production and utilization. Oxidative phosphorylation must, apparently, be intact for iodide transport to occur. Various agents that uncouple oxidative phosphorylation, such as 2,4-dinitrophenol or dicumarol, depress the T/M both for iodide and for certain other anions that are concentrated by thyroid tissue (91, 287, 352). It is, of course, not certain whether these are effects on ATP or on some precursor high-energy intermediate. Apparently not enough ATP is generated by glycolysis, substrate phosphorylations, etc., to permit iodide transport under anaerobic conditions.

If we assume that ATP is, in fact, required, I^- transport could be inhibited either by interfering with ATP production (as with the uncoupling agent listed above) or by interfering with ATP utilization. Ouabain and other cardiac glycosides appear to act on the latter process. Cardiac glycosides prevent the transport of both cations and anions, and recent evidence supports the view that this inhibition operates via an effect on a Na^+-K^+-requiring ATPase activity sensitive to these drugs. Wolff and Halmi (345) have shown that such activity is present in the thyroid and also in the other iodide-concentrating tissues. A remarkable correspondence in the transport and ATPase systems exists for: *1*) ouabain sensitivity of thyroid of various species; *2*) the potency of various glycoside analogues; *3*) half-saturation with K^+; 4) reversal of ouabain-inhibition by K^+; 5) the response to TSH. The presence of this ouabain-sensitive, Na^+-K^+-requiring ATPase activity has been demonstrated independently in beef thyroid preparations. It was stimulated in vitro with large doses of TSH (312). While the thyroid gland contains much other ATPase activity the effect of TSH is primarily on the ouabain-sensitive, Na^+-K^+-activated system (Fig. 3). The identity of the ATP-utilizing system has not been discovered, but may possibly involve the synthesis and breakdown of a phosphorylated carrier.

B. Effect of Temperature

The ability of thyroid slices to concentrate iodide is markedly diminished at low temperatures (90, 287). In preliminary experiments we have found a Q_{10} of 2.4 for the T/M[I^-] in sheep thyroid slices. It is of interest that such slices incubated at 30 C attained the same T/M[I^-] as those at 37 C; however, equilibration took longer at the lower temperature (Wolff, unpublished observations). The Q_{10} for I^- transport by the isolated choroid plexus was found to be 2.1 (26).

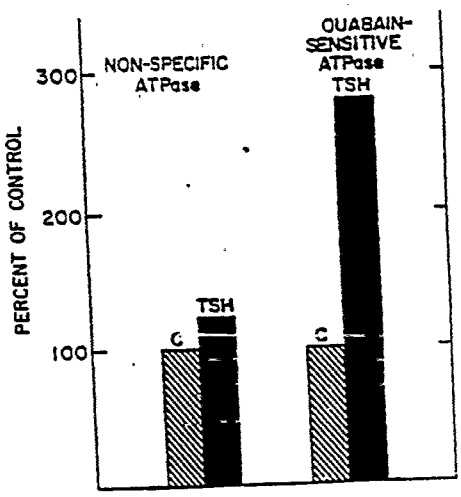


FIG. 3. Effect of thyrotropin (TSH) on the ATPase activities of the guinea pig thyroid. Comparison of the relative degree of ATPase stimulation in homogenates of pooled glands from animals treated with 1.0 IU of TSH per day for 3 days. Nonspecific ATPase activity is defined as the activity remaining in the presence of a high concentration of ouabain ($5 \cdot 10^{-4}$ M). C = controls, TSH = thyrotropin-treated guinea pigs (based on data from ref. 345).

The rate of drop in the T/M[I^-] on exposure to a lowered temperature is sufficient to suggest that some values in the literature should probably be higher than reported (308). Because of the rapid loss of I^- from thyroid tissue exposed to cold (e.g. 90), extreme care should be taken that such exposure is minimal or avoided entirely. Failure to do this may lead to low T/M[I^-] values.

C. Sulfhydryl Groups

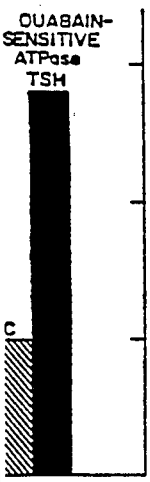
Several investigators have shown that heavy metals and alkylating agents which react with —SH groups inhibit iodide transport (91, 287). It is interesting that the T/M[I^-] is more sensitive to these reagents than is the QO_2 (91), but so many tissue proteins require functional —SH groups that such experiments have offered few clues. Interpretation is complicated by the fact that —HgI complexes or compounds might form. The effect of Hg compounds is reversed by cysteine (91, 287), and a variety of compounds with reactive —SH groups, or —S—S- groups that are easily reduced, increase the T/S[I^-] in vivo. This includes cysteamine, penicillamine, and 2,2′-dithiobisethylamine (287, 350). It is of interest that the Na^+-K^+-ATPase activity of brain is sensitive to —SH reagents (286).

D. pH Optimum

The pH optimum for I^- concentration in thyroid slices is 7.5 to 7.8 (in external medium) (90, 287). Since the intracellular pH of thyroid tissue may be lower (69), it cannot be stated at present whether the effects of deviations from this optimum are related to proton-cation exchange or are secondary to effects on metabolic processes.

E. Cation Requirement

Deletion of Na^+ or K^+ from Ringer media in which thyroid slices are incubated (90) or leaching iodide-concentrating tissues with K^+-free Ringer medium



lowers the T/M[I^-] (337, 347). This decrease is restored by addition of K^+. Half-maximal stimulation occurred at 0.9–1.4·10^{-3} M K^+ added to the medium. On the basis of the findings with the thyroid gland (337) and salivary and mammary tissue (191a, 347), other investigators have shown a similar K^+ requirement for I^- accumulation by the choroid plexus (26, 329).

The ouabain-induced depression of the T/M[I^-] is partially reversed by K^+ (or Rb^+) (337, 347). Since the half-saturation values for K^+ are the same as those for the thyroidal Na^+-K^+-activated transport into various cells (e.g. 100, 163, 270, 290), the tentative conclusion has been reached that K^+ or Na^+ transport is a prerequisite for I^- transport. The cardiac glycosides inhibit K^+ (Na^+) transport and this inhibition in turn prevents the transport of iodide. The Na^+-K^+ pump (and Na^+-K^+ ATPase) is present in nearly all mammalian cells, and we have suggested (345) that transport of many substances such as iodide (337, 347), *p*-amminohippurate (50), amino acids (28, 166, 184, 224), and various sugars (62, 64, 167, 247) is linked, in some manner peculiar to each tissue, to this fundamental transport system of the cell. Woodbury and Woodbury (375) have come to similar conclusions from an entirely different experimental approach. It is of interest that TSH injections cause a rapid increase of thyroidal Na^+ and K^+ in guinea pigs and chicks (98, 291). This may be associated with the rise in QO_2 of thyroid slices that is inhibited by ouabain (312). Since the T/M [I^-] is not increased by adding TSH in vitro, the significance for iodide transport of this fraction of the respiration is not clear.

VI. INHIBITORS

Substances that inhibit iodide transport can be classed into three categories: *A*) metabolic inhibitors, *B*) transport inhibitors, and *C*) competitive anions.

A. Metabolic Inhibitors

The metabolic inhibitors are, in part, discussed in the section on energy requirements. In addition to the well-known respiratory poisons and uncoupling agents, this group contains all the SH-reagents so far tested and numerous quinones. Most of these agents lower the QO_2 at the same time that they depress the T/M[I^-], although increased QO_2, presumably due to uncoupling of oxidation phosphorylation, occurred with decreased T/M[I^-] in the presence of a number of nitrophenols (90, 91, 287). The lack of selectivity permits few conclusions besides the fact that aerobic metabolism and energy production must be intact for I^- transport.

B. Specific Transport Inhibitors

Of the inhibitors believed to act more or less specifically on transport processes, the cardiac glycosides or their aglycones, quinidine and phloridzin, have been tested. The evidence that ouabain and other cardiac glycosides act as specific transport inhibitors rather than as metabolic poisons is suggestive but as yet

incomplete.[3] It seems safe to say, for the present, that these compounds are relatively specific inhibitors of ion transport, acting probably via an inhibition of the Na^+-K^+-requiring ATPase activity, which controls K^+ (and Na^+) transport, and loss of which in turn depresses iodide transport (337, 345–348). Radioautographs of dog thyroids show marked concentration of ^{14}C-labeled lanatoside C in the cells (but not the lumen) of the gland (43). Tadpoles exposed to strophanthin have delayed metamorphosis despite increased growth. There is no delay if thyroxin is also given (140).

On the basis of the finding that acetazolamide (Diamox) inhibits ^{131}I uptake by the thyroid gland in vivo, it was suggested that carbonic anhydrase was involved in iodine uptake (95). The enzyme has been demonstrated in the thyroid, is sensitive to acetazolamide (171), and a report has appeared that acetazolamide interferes with I^- transport in vivo (374). No such effect was found in the better defined in vitro system (91). We have confirmed this negative result at concentrations a thousandfold greater than required to inhibit carbonic anhydrase (unpublished). The inhibition of ^{131}I uptake is probably due to the antithyroid properties shared by many sulfonamides.

Phloridzin and phloretin depress the T/M[I^-] at rather high concentration ($\sim 10^{-3}$ M) (91) (Wolff, unpublished). Since ATP hydrolysis is inhibited by these agents (175), the mechanism may be similar to that of the cardiac glycosides.

Quinidine depresses the T/M[I^-] of sheep thyroid slices (287, 337) and also inhibits the Na^+-K^+ ATPase believed to be involved indirectly in iodide transport (337, 345). K^+-reversal of this effect has not yet been demonstrated. However, this compound is also a strong inhibitor of mitochondrial (rabbit heart) respiration (117, 287), and its action may well be complicated.

C. Competing Anions

Evidence often taken to indicate active transport is the competitive inhibition by chemically related substances which have similar properties regarding saturation, effects of inhibitors or activators, temperature, etc. A number of anions have been found to fulfill such a role, and the present section deals with their properties. The reader may find this to resemble a rag-bag of haphazard observations. To a certain extent it is. We have therefore organized the findings to answer, if possible,

[3] At low concentrations, cardiac glycosides inhibit cationic fluxes without depressing respiration or glycolysis. At high concentrations, respiration may be inhibited (109, 117, 172, 178, 270, 331, 354). Changes in high-energy phosphate levels occur with doses well in excess of those required to produce depression of cation fluxes (94, 116, 178, 219, 354). Furthermore, glycoside effects, such as on cardiac contractility, occur before changes in either oxygen consumption or high-energy phosphates (121, 178). Oxidative phosphorylation is not influenced by $3.3 \cdot 10^{-4}$ M digitoxin in liver or heart mitochondria. The uncoupling action of dinitrophenol is, however, potentiated by digitoxin (109, 117, 273). Ouabain or digitoxin enhance labeling of phospholipids in homogenates of various tissues (195, 219, 383) but may also inhibit. In thyroid slices, digitoxin inhibits $P^{32}O_4$ incorporation into phospholipids—an effect partly reversed by K^+ ions (363). This difference between broken and intact cells is probably an important consideration when evaluating biochemical mechanisms of cardiac glycosides. There is no effect of ouabain on adenosine deaminase (216), nucleotide labeling (109), or glutamine synthetase (109), and several K^+-activated enzymes are not influenced by ouabain (337).

the following three questions: *a*) does the anion inhibit iodide transport? *b*) is it itself concentrated by thyroid tissue? and *c*) what is the mechanism of its action? Most of the studies have dealt with the inhibitory effect of the anions on iodide transport since this is the easier experiment to carry out.

A number of special factors must be considered in evaluating the data, and these have, unfortunately, not always been secured. They are:

1) A number of the anions are not perfectly stable under the test conditions (e.g. OCN^-, IO_3^-, SCN^-, $SeCN^-$, etc.) (5, 177, 194, 349), and their metabolism must be taken into account.

2) Many of these anions are bound to proteins to a greater or lesser extent (54, 267, 268, 349, 356), and this will influence calculations of their K_m or K_i values, etc.

3) The conditions for attaining I^- equilibrium may be altered by the simultaneous presence of these anions. Again, this possibility has been considered only occasionally (349, 356). Most of these anions work on extrathyroidal as well as thyroidal transport mechanisms, but unless special points are to be illustrated, these are not discussed since they have recently been reviewed (46). The following references should, however, be added for completeness: 25, 26, 120.

Except for F^-, Cl^-, and $SO_3NH_2^-$, the univalent inorganic anions described here are either inhibitors of iodide transport, are themselves concentrated, or both. Thiocyanate ion differs in that it inhibits without being concentrated above the levels of the medium.

1. The halides. A. FLUORIDE. It is largely because of the widespread use of water fluoridation that interest has been displayed in possible effects of F^- on the thyroid gland. As long ago as 1926 it was pointed out the F^- might be goitrogenic (106), and the coexistence of fluorosis and goiter in some areas has been cited as evidence for F^- as a cause of endemic goiter (298, 334). In general, however, experiments in man or animals with F^- levels that might be attained in fluoridation have shown no effect of this anion on thyroid function (68, 99, 145, 183). A considerable amount of confusing clinical work deals with the effects of NaF in thyrotoxicosis. The results are inconsistent (96, 147). In those cases in which fluoride was claimed to have antithyroid properties the mechanism was not investigated. Inhibition of the coupling of iodinated tyrosines was claimed in a single report (299).

Fluoride concentration by the thyroid has been reported in only a single instance, and that by a rather difficult chemical method (55). When $^{18}F^-$ has been used, there has been no concentration of the isotope in the gland (7, 146, 328). High F^- concentrations in salivary and gastric secretions have been reported (146, 328, 336), but salivary/serum ratios have been variable, with a few reports of ratios near 1.0 (53, 146, 328, 333). Where measured, the ratio for F^- was less than that for Cl^- (333).

Depression of I^- transport in salivary gland, choroid plexus, and ciliary body is reported to occur at $1 \cdot 10^{-3}$ M F^- (25, 26, 84). Others have been unable to inhibit thyroidal iodide transport at $3 \cdot 10^{-3}$ M F^- (91, 347). Since large doses of F^- may have numerous other effects (e.g. on glycolysis), it seems safe to conclude that no

unequivocal evidence has been produced for an F^--concentrating mechanism in thyroid tissue.

B. CHLORIDE. The chloride ion has not, so far, been found to depress the T/S[I]. Thyroid tissue contains more Cl^- than do a number of other tissues (20, 188). The level does not exceed serum concentrations and appears to be, in part, a function of the extracellular space (lumen) in the gland. More elaborate studies with $^{24}Na^+$ have also led to the conclusion that the thyroid gland contains additional extracellular space which equilibrates rather more slowly than that of other tissues (291). The Cl^- content is increased in parallel with the water content at early intervals after administration of TSH and then returns to normal (188, 315). Woodbury and Woodbury (375) have calculated that the thyroid chloride concentration, as determined from the Cl^- space, is in good agreement with that calculated from measurements of the electrical potentials (18–19 mM). This suggests passive distribution of Cl^-.

An indirect effect of Cl^- ion on thyroid function can be shown. A moderately low-iodine diet supplemented with large Cl^- concentrations is goitrogenic, depletes thyroid iodine, and increases the avidity of the thyroid for $^{131}I^-$ (14, 148, 158, 159, 245, 332, 382). Acute treatment with chloride lowers the thyroid uptake of I^{131}. This effect appears to be on the kidney, which is unable to differentiate the two halides well enough to prevent iodide loss in the face of a chloride load (129, 159). A common tubular transport system for monovalent inorganic anions may exist (129, 237, 302) but present evidence is incomplete. Certain cations [Na^+, K^+, Rb^+ but not Li^+, Ca^{++} or NH_4^+] also increase urinary iodine output (158).

C. BROMIDE. Claims have been made that chronic bromide treatment lowers the thyroid iodine content, the metabolic rate, and the growth rate (208, 209, 285). Bromide ion has also been stated to be goitrogenic and to interfere with the uptake of $^{131}I^-$ (61). It is likely that a number of these effects are toxic reactions to this halide, and no Br^- effects have been found in short-term experiments (313, 379).

Much of the early work on the relation of bromide to the thyroid gland was based on inadequate chemical analyses. However, the thyroid gland has the highest concentration of Br^- of any organ in the mammalian body (23, 208). The thyroid gland can establish a small concentration gradient of $^{82}Br^-$ over plasma, i.e. $T/S[Br^-] > 1$, although a negative report has also appeared (1, 22, 193, 226, 254, 380). Concentration occurs in the presence of thiouracil or in its absence (19); no organic Br is formed (380), perhaps because the redox potentials for iodine and bromine differ markedly. The ratios for gastric juice and saliva are near 1.0, the ratio of the rate coefficients for secretion of Br^- and Cl^- is reported to be 1.53 (146a), but data are conflicting at present (193, 217, 380). It seems safe to conclude, however, that the thyroid can concentrate bromide, albeit weakly. Large concentrations of bromide inhibit iodide transport in vitro in sheep thyroid slices (91, 349) or in the choroid plexus (26) ($2 \cdot 10^{-3}$ M and $5 \cdot 10^{-4}$ M, respectively, for 50% inhibition). The inhibition is competitive in reciprocal plot analysis (349). Bromide also increases the clearance of iodide by the kidney in rats (129).

D. ASTATIDE. The heaviest member of the halogen series exists only in the

radioactive form. For biological purposes the most useful isotope is $^{211}_{85}$At (half-life 7.5 hr). Astatine is more metallic than iodine but otherwise resembles it in many respects (e.g. solubility, volatility, redox potential, etc.) (153). Its uptake by the thyroid was studied as long ago as 1940 (144) (At was then called ekaiodine). The uptake is less than that of iodide in the unblocked thyroid but greater in the presence of thiouracil (279). It is depressed by simultaneous KI administration, enhanced on a low-iodine diet (141, 142), increased by other thyroid stimulation (73, 144, 279), and decreased by thyroxin treatment (278). Values for the T/S[At⁻] are in the neighborhood of those for iodide but conditions for these experiments have often not been identical or have failed to account for At not present as the anion (73, 141, 143, 278). Although the thyroid and stomach contain much higher amounts than other tissues, nearly all organs except muscle concentrate the isotope more than plasma. Because of this and the chemical behavior of At at pH 7 (141):

$$\begin{array}{ccccccc} & & At_2^0 & & & & \\ & & \downarrow\uparrow & & & & \\ At^- & \rightleftharpoons & At^0 & \rightleftharpoons & AtCH & \rightleftharpoons & AtO^- + H^+ \\ & & & & & & \downarrow\uparrow \\ & & & & & & At^+ \end{array}$$

it is possible that some of these gradients are established by forms other than the anion. The redox potential of the astatide-astatine couple is 0.4 v; hence an oxidation would be possible in the thyroid (153), but oxidized or organic forms have not been identified.

A sizable portion of thet $^{211}_{85}$A of the thyroid is, nevertheless, present as the anion. The findings are: *1*) At⁻ accumulation is not blocked in the presence of propylthiouracil (73, 279); *2*) KSCN prevents the accumulation of At⁻ and can discharge a portion of previously accumulated At (278) (however, if reduction is sufficiently rapid only a small proportion of the total At would actually have to be present as the anion at any time); *3*) about half the thyroidal ^{211}At is soluble in 80% ethanol (unlike protein-bound iodine) (134). It may thus be concluded that the astatide anion is concentrated rather well by thyroid tissue.

2. Pseudohalogens. Into this group fall certain univalent compounds of two or more electronegative atoms which possess certain characteristics of the halides, and, in the free state, resemble the halogens. Included are CN^-, OCN^-, SCN^-, $SeCN^-$, $TeCN^-$, $SCSN_3^-$ (azodithiocarbonate), N_3^-, and ONC^- (fulminate). Cyanide and probably azide have special properties that make evaluation of specific transport properties difficult. Tellurocyanate, ONC^-, and $SCSN_3^-$ are not sufficiently stable for ready use in these systems but OCN^-, SCN^-, and $SeCN^-$ have been evaluated for their role in anion transport in the thyroid and certain other tissues.

A. THIOCYANATE. The antithyroid properties of anions were first discovered as a side effect in the treatment of hypertension with SCN^- (17). Some three dozen cases of goiter and/or hypothyroidism have been described in such patients (18, 31, 78, 86, 169, 233, 238, 246). Clinical and chemical evidence has demonstrated an interference with iodine metabolism, leading to hyperplastic, iodine-

poor goiters with a depressed iodine uptake when the level of the drug is high. Astwood (11) made similar observations in the rat and found, in addition, that iodide supplements prevented the SCN^- goiters. These findngs have since been amply confirmed (201, 263, 332).

More detailed investigations on the nature of the thiocyanate effect revealed that this anion inhibits the accumulation of inorganic iodine both in tissue slice experiments (87, 344) and in vivo (316, 344, 358). This effect persists in the gland blocked by propylthiouracil. The blocking action of SCN^- depends on the presence of high concentrations of SCN^- (344, 356) and disappears as soon as the blood level has fallen sufficiently. In fact, in glands of chronically treated animals, thyroid hyperplasia is sufficient to increase ^{131}I uptake provided most of the SCN^- has left the circulation (31, 237, 240, 332, 344).

An important extension of the concept that SCN^- acts on the iodide transport came from studies of the brothers VanderLaan (313). They showed that accumulated I^- could be rapidly discharged from the propylthiouracil-blocked thyroid of rats with an injection of SCN^-. The discharged iodine was identified as the anion. This purging action of SCN^- proved to be an excellent index of the degree to which antithyroid therapy or genetic defects have blocked the conversion of inorganic iodine to organic forms (47, 88, 239, 293, 297, 313, 352). That is, this action of SCN^- (and certain other anions) has been used as "proof" that a portion of thyroidal iodine is the anion. It is also used to test for iodide concentration in other organs such as salivary gland or saliva, mammary gland or milk, skin and hair, stomach and gastric juice, ciliary body (25), choroid plexus (26), and the fetal thyroid (262). Studies on most of these organs have been reviewed recently (46).

On the assumption that the SCN^- effect on iodide trapping was competitive in nature, numerous investigators have sought to show SCN^- accumulation in thyroid tissue. It is known that certain other tissues that accumulate iodide ion can also accumulate SCN^-. Thus SCN^- is significantly concentrated in saliva and gastric juice (83, 85, 189). Fletcher et al. (85) found T/M[SCN^-] values in mouse salivary glands $\simeq$ 10. Much of this SCN^- ion was not exchangeable with I^- or ClO_4^-. There is, however, no question from work with labeled SCN^- that this anion *is* concentrated in these tissues (189, 190). Clear-cut evidence for SCN^- concentration by the isolated choroid plexus has been published (329). This process is remarkably similar to I^- concentration in the thyroid and in this organ, showing inhibition by ClO_4^-, BF_4^-, and I^-, by metabolic inhibitors such as dinitrophenol, F^-, or CN^-, and by the transport inhibitor digoxin. Furthermore, the system shows saturation kinetics. The ability to transport SCN^- leads to movement *out* of the cerebrospinal fluid (232a), as in the case of I (26, 329).

With regard to thyroidal SCN^--concentrating ability, much confusion has arisen from failure to define terms. If "concentration" is to mean a T/S[SCN^-]>1.0, then no concentration has so far been observed (191, 363, 372, 373). A single report of T/S[SCN^-] values >1 has been published (20). Since it involves a technically cumbersome method, the results with labeled SCN^- would seem to be the more reliable. On the other hand, any T/S[SCN^-] value greater than the

"diffusion" space would be significant. This has not been determined. Radioautographic evidence shows localization of radioactivity from $^{35}SCN^-$ in the lumina of some thyroid follicles (60, 191), although the T/S[SCN^-] was <1.0. Most of this activity was present as SCN^-.[4] Since the anion transporting systems of other mammalian tissue are so remarkably similar to the thyroid in all respects other than SCN^- concentration (25, 26, 46, 329, and Tables 1 and 2), the lack of readily demonstrable SCN^- concentration by the gland may merely represent the additional ability of this organ to remove thyroidal SCN^- by metabolism or a rapid efflux mechanism. Such a postulate requires experimental verification.

Wollman (356) showed that SCN^- is a competitive inhibitor for iodide transport in the intact mouse. That is, it influences the K_m for I^-, not the A function. Competitive inhibition has also been shown in sheep thyroid slices (349). The K_i for SCN^- is from 2 to $6 \cdot 10^{-5}$M (349, 356); it appears to be similar in other iodide-concentrating tissues as well (25, 26, 85, 329). Analysis of equilibration curves has yielded the surprising result that in the blocked gland SCN^- increases the K_{TB} (29, 360). The resulting K_{TB} values are often very much greater than those in the absence of SCN^- and raise some doubts about the simple diffusion concept of the exit process of I^-. The interpretation of the change in K_{TB} differs if analysis is made according to a two- or a three-compartment model (360). In this model the effective exit rate constant can be increased either by inhibition of cell-to-lumen iodide transport or by directly increasing cell-to-blood transport. Under different experimental conditions, a primary effect on the clearance has been reported (128), and in the unblocked rat gland a better fit with the data is obtained if it is assumed that the effect is on clearance (358). Suffice it to say that analysis of the locus of SCN^- action cannot be made with confidence at present.[5]

From time to time investigators have considered the possibility that SCN^- may interfere with the oxidation of iodide as well as its transport (55, 87, 234, 344). This seemed very reasonable on chemical grounds. The oxidative system of mammalian thyroids can oxidize at the potential of the iodide couple but not at the bromide couple. The SCN^- couple is nearer iodide and hence would seem a likely candidate for oxidation:

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[4] With all the more complex anions the possibility that they are degraded must be considered in evaluation of their effects. SCN^- is metabolized to $SO_4^=$ (191, 194, 372, 373). The proportion of SCN^--^{35}S as sulfate increases with time, and may be ⅔ of the total at 5 hr. Degradation is inhibited by thiourea, propylthiouracil, iodide and sulfadiazine (194, 373). Thus studies in glands blocked with a thiocarbamide drug will be relatively free from this complication. A slight stimulation by TSH of SCN^- degradation has also been reported (266).

[5] The suggestion has occasionally been made that the inhibitory effect of SCN^- results from nonspecific toxic effects. It has been proposed that the anion inhibits respiratory enzymes in a manner not unlike cyanide (93, 107, 221); it inhibits oxidation of amino acids by liver and kidney (221, 301), or uncouples oxidative phosphorylation (164). Thiocyanate also combines with certain heme compounds (162) and inhibits carbonic anhydrase (66). The latter is of special interest because this enzyme has been claimed to play a role in iodide transport (84); these findings have not been confirmed. In most of these cases more SCN^- is required than is necessary to inhibit iodide transport, and competitive kinetics make these suggestions seem unlikely.

	E°_{298}
$I^- \rightleftharpoons \frac{1}{2}I_2 + e^-$	$= -0.535$ v
$SCN^- \rightleftharpoons \frac{1}{2}(SCN)_2 + e^-$	$= -0.77$ v
$Br^- \rightleftharpoons \frac{1}{2}Br_2 + e^-$	$= -1.07$ v

Furthermore, iodide interferes with the degradation of SCN^- by thyroid tissue (194), and SCN^- in turn inhibits iodination in cell-free systems (4, 228, 307, 376) In intact cells, however, the inhibition could be due to rate changes alone—resulting from decreased substrate concentration. Kinetic analysis (358) has shown that at low serum SCN^- levels inhibition of I^- transport accounts for nearly all of the SCN^- effect. At high SCN^- levels a decreased fraction of thyroidal iodide was incorporated into organic iodine, but differentiation between a substrate or enzyme effect could not be made.

Thiocyanate enjoys a special place among the antithyroid anions because it is the only one that might be involved as a natural goitrogen. The evidence for such an action is far from satisfactory, however. The two analytical methods most often employed for SCN^- determinations are both rather nonspecific and many interfering substances may be found, especially in materials of animal origin (202). Thus phenols, keto acids, etc., may interfere with SCN^- determination by the Fe^{+++} method, whereas tyrosine, tryptophan, methylthiouracil, etc., interfere with the cyanogen bromide method (3). If allowances are made for these shortcomings the following rough conclusions seem justified. It has been held for many years that most of the SCN^- in the body arises from the detoxification of CN^-, which in turn is derived from organic nitriles or cyanogenic glycosides (e.g. amygdalin). However, recently thioglycosides called glucobrassicin and neoglucobrassicin have been isolated from various types of cabbage. The enzyme myrosinase liberates SCN^- from these compounds (101, 102, 160, 174, 323), and up to 300 mg may be liberated from 1 kg of cabbage (101, 323). It was considered possible that a high cabbage intake in a rather low-iodine area could be goitrogenic. Attempts have also been made to correlate the level of serum SCN^- with the presence of simple or toxic goiter in man (33a, 244, 280). The determination of serum SCN^- (15, 151) is subject to the errors mentioned above, and even the method of Boxer and Rickards (40) (which also depends on oxidation of SCN^- to CN^-) gives normal serum concentrations (40, 220) which, at their lowest, amount to 1 to $2 \cdot 10^{-4}$ M. Although much of this will be bound to serum albumin, substantial thyroid inhibition would be expected. (It is of interest that SCN^- concentration in gastric juice was ~10-fold greater than the serum while cerebrospinal fluid was ~10-fold less). Thus the data for goiters produced by SCN^- from natural sources are not very persuasive so far. Control of I^- transport by SCN^- does, however, seem an attractive possibility and its role merits further study.

In conclusion, it has been established that the SCN^- ion inhibits the iodide-concentrating mechanism competitively with respect to iodide, without being itself accumulated to a significant extent. This raises special kinetic problems that have not been fully evaluated (128, 349, 356, 357, 360).

B. CYANATE AND SELENOCYANATE. Of the other pseudohalides the next lower

(OCN^-) and next higher ($SeCN^-$) homologues of SCN^- have been investigated with regard to thyroid function. Tellurocyanate has not been available, and is apparently too unstable. Both OCN^- and $SeCN^-$ have been used only in vitro (349, 352): cyanate is a weak inhibitor of iodide transport whereas $SeCN^-$ is of about the same potency as SCN^-. The $SeCN^-$ ion can be concentrated by thyroid tissue of rats and guinea pigs, mouse submaxillary tissue and rat thyroid tumor (Maurey and Wolff, unpublished). Preliminary evidence suggests, however, that the concentrating process may differ from that common to the other univalent anions.

3. Nitrate. Nitrate ion has been shown to be a weak inhibitor of iodide transport in rat and sheep thyroids in vivo, sheep thyroid slices, human salivary glands, and salivary slices of mice (32, 74, 85, 91, 349, 378, 379). It is goitrogenic and can discharge previously accumulated I^- from the thyroid (379). The K_i (50% inhibition) values in sheep thyroid slices (349), rabbit ciliary body (25) and rabbit choroid plexus (26, 329) are about 20- to 100-fold that of SCN^-. It has been suggested that part of the goitrogenicity of NO_3^- resides in its ability to increase renal clearance of iodide (319). While this may contribute, there can be no doubt that NO_3^- exerts a direct thyroidal effect against iodide transport. No studies of concentration of NO_3^- by iodide-concentrating tissue have been carried out.

4. Complex anions. The remaining anions related to iodide transport comprise a series of univalent, polyoxy-anions as well as some in which F^- may substitute for one or more of the oxygen atoms. They are: ReO_4^-, ClO_4^-, TcO_4^-, and SO_3F^-, $PO_2F_2^-$, and BF_4^-. Certain other complex anions such as ClO_3^-, IO_3^-, and IO_4^- were at one time included in this group, but there is reason to believe that they are too unstable and are reduced in the test systems (177, 254, 349). This holds also for BrO_3^-. The effective anions are of the body-centered tetrahedron type with oxygen or fluorine atoms occupying the four corners.

A. PERCHLORATE. Wyngaarden and coworkers (378, 379) first showed that perchlorate ions could prevent accumulation or retention of ^{131}I in the rat thyroid, cause thyroid enlargement, and discharge accumulated ^{131}I in thyroids of animals treated with propylthiouracil. Perchlorate is from 10 to 100 times as potent as SCN^- in a variety of in vivo and in vitro test systems (25, 85, 91, 156, 349, 378). Iodide distribution in tissues that do not concentrate the halide is not significantly influenced by ClO_4^- (138). The high potency of perchlorate led to its trial for the treatment of hyperthyroidism (63, 103, etc.), where it now is accepted therapy. Perchlorate is excreted essentially unchanged; in the rat approximately 90% is eliminated in 24 hours, and the rate of its appearance in the urine can be accelerated by iodide or SCN^- (6, 77). This rapid excretion is also reflected in the recovery of the I^{131} uptake 24 hours after withdrawal of ClO_4^- (80). In man indirect evidence suggests that the turnover of ClO_4^- is much slower (108, 139).

It has been shown recently that radioactivity from ^{36}Cl-labeled ClO_4^- is concentrated in the thyroid gland and not in other tissues (6). Because of the low specific activity attainable with this isotope the $T/M[ClO_4^-]$ was low, i.e., the system was probably nearly saturated. The $T/M[ClO_4^-]$ could, nevertheless, be doubled by pretreatment of the rats with thyrotropin (185). If the radioactivity

in the thyroid exists truly as ClO_4^-, and this has not yet been demonstrated, higher values of the $T/M[ClO_4^-]$ may be expected if material of higher specific activities are made available.

Unlike thiocyanate ion, ClO_4^- interferes only slightly with the formation of organic iodine in cell-free systems (4, 67, 309, 376). Data so far available suggest that the ClO_4^- effect on iodide transport is competitive, with a K_i of $4 \cdot 10^{-7}$M (349). The effect is primarily on iodide clearance (128).

Iodide transport in all the other iodide-concentrating tissues so far tested is inhibited by ClO_4^- in vivo and in vitro, and this agent has become as much of a standard tool as SCN^- for elucidation of the iodide-concentrating mechanism in these tissues (e.g. 25, 26, 46, 125, 329).

B. PER-ANIONS OF PERIODIC GROUP VII A. The VIIth periodic group is divided into the halides (VII B) with an outer electron configuration of 2 s and 5 p electrons, and group VII A of the transition series (Mn, Tc, Re) with an outer electron configuration of 5 d and 2 s electrons. The anions with which we are concerned are MnO_4^-, TcO_4^-, and ReO_4^- (70, 241). Mn^{++} is concentrated in the thyroid but less so than in a variety of other tissues (70, 200, 241). Surprisingly, the amount in the thyroid in relation to other organs increases with increasing amounts of carrier (49). The concentration of Mn^{++} differs from that of the anions in that it does not require cellular integrity, metabolic energy, or K^+ (348). In only one case has MnO_4^- been studied (22)—its behavior was stated to be identical to Mn^{++}, suggesting that reduction had occurred. Such reduction can easily be observed in systems in vitro (348), as might be expected from its high redox potential. Thus inclusion of MnO_4^- in this group is hypothetical.

Technetium exists only in the radioactive state, but isotopes of sufficiently long half-life (^{99}Tc = $2.12 \cdot 10^5$ yr) exist to have permitted reasonably good chemical identification of its compounds (41). Pertechnetate, unlike permanganate, is stable over a wide pH range and, in general, resembles ReO_4^- more than MnO_4^-. Pertechnetate ion is concentrated by rat, sheep, mouse, and human thyroid tissue, by rat thyroid tumor slices, and in mouse salivary slices (9, 10, 22, 348). It remains largely in the chemical form TcO_4^- (9, 348).

Perrhenate is concentrated to a similar extent (24, 254, 277, 348); it also is not chemically altered (348), which is not surprising in view of an even lower redox potential than TcO_4^-. Shellabarger (277) has, however, found some ^{188}Re in the "protein" fraction of thyroid glands. Comparison with iodide accumulation must be made in the goitrogen-blocked thyroid, and either with carrier-free materials or at the same concentration relative to the K_m. With high specific activity $^{99}TcO_4^-$ in mice, the $T/S[TcO_4^-] > T/S[I^-]$; this was also true in sheep thyroid slices with concentrations adjusted to the K_m (9, 348). In older studies, $T/S[ReO_4^-]$ values were reported as less than the $T/S[I^-]$ values (24, 277). With concentrations adjusted to the K_m values, $T/M[ReO_4^-] > T/M[I^-]$ in thyroid slices from the same sheep (348). The K_m values (half saturation) are $3\text{–}5 \cdot 10^{-7}$M for TcO_4^- and $1 \cdot 10^{-6}$ for ReO_4^- compared to $3 \cdot 10^{-5}$M for I^-.

As with I^-, transport of these anions requires cellular integrity, metabolic energy, and K^+ ions in the medium. Any one ion can inhibit the concentration of

TABLE 4. *Relation of K_m and K_i to anion size (Φ_0)**

Anion	K_m, M	K_i, M	Partial Molal Vol., Φ_0 at 25 C, cc/mole†
Bromide (Br^-)		$\sim 2 \cdot 10^{-2}$	25.1
Cyanate (OCN^-)		$1-2 \cdot 10^{-2}$	26.7
Nitrite (NO_2^-)		$4 \cdot 10^{-3}$	25 (20C)
Nitrate (NO_3^-)		$1-2 \cdot 10^{-2}$	29.4
Iodide (I^-)	$3 \cdot 10^{-5}$, ($2.6 \cdot 10^{-5}$‡)		36.7
Astatide (At^-)			40.2§
Thiocyanate (SCN^-)		$2-3 \cdot 10^{-5}$, ($6 \cdot 10^{-5}$‡)	40.6
Monofluorosulfonate (SO_3F^-)		$1-2 \cdot 10^{-5}$	47.8
Selenocyanate ($SeCN^-$)		$1 \cdot 10^{-5}$	50.3
Tetrafluoroborate (BF_4^-)		$3 \cdot 10^{-6}$	44.0
Perrhenate (ReO_4^-)	$1 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	48.7
Perchlorate (ClO_4^-)		$4 \cdot 10^{-7}$	44.5
Pertechnetate (TcO_4^-)	$3-5 \cdot 10^{-7}$		46.0

* From Wolff & Maurey (349). † Defined as volume of anion occupied in solution extrapolated to 0 concentration. ‡ In vivo measurements (356, 365). § Estimated by extrapolation of straight-line relation between anionic radii and Φ_0 for halides. Radius of At^- was taken as 2.27 A (275).

any other with a potency determined by the K_m values. A small but significant binding of TcO_4^- and ReO_4^- by concentrated homogenates of thyroid tissue has been found (348). This is in contrast to the behavior of I^- (343). Preliminary experiments with highly purified porcine thyroglobulin (>99% 19S material) showed only minimal concentration of TcO_4^- or ReO_4^-, even at protein concentrations of 2.2 % (Wolff, unpublished). Thus it can be concluded that these three ions share, in large part, the same transport mechanism.

Several other complex anions (BF_4^-, SO_3F^-, $PO_2F_2^-$) have been studied because of their special properties and are discussed in the next section (5–7, 26, 185, 329, 349).

In conclusion: the tetrahedral anions ClO_4^-, TcO_4^-, ReO_4^-, and BF_4^-, are concentrated by thyroid glands, and are probably not significantly metabolized there. In addition they, as well as SO_3F^- and $PO_2F_2^-$, also inhibit iodide accumulation (or retention, where tested). The K_m and K_i values, tested in sheep thyroid slices or in the intact rat, are listed in Table 4.

VII. SIZE REQUIREMENT

On the assumption (6, 11, 22, 201, 263, 277, 332, 348), and later the proof (349, 356), that the halides, pseudohalides, and tetrahedral anions are competitive inhibitors of iodide transport, various attempts have been made to find *that* property which established their kinship in this system.

Univalency appears to be a requirement for anions of this group. Divalent anions such as SO_3^-, SO_4^-, $S_2O_3^-$, $S_2O_8^-$, HPO_4^-, WO_4^-, and MoO_4^- do not inhibit iodide transport at relatively high concentrations, and SO_4^- is not concentrated by thyroid tissue (91, 194, 349, 379). While univalency appears to be a necessary con-

dition for thyroidal anion transport, it is not a sufficient condition. In addition F^- and Cl^-, mentioned above, the following anions are not inhibitory for iod. transport: formate, acetate, sulfamate, methylsulfonate, phenylsulfonate, a.. ascorbate (349, 379).

The suggestion that anions affecting the thyroid are placed near iodide in the Hofmeister or lyotropic series was first made by Baumann and Metzger (20) and later by Wyngaarden et al. (379). This series comprises the following anions listed in order of increasing lyotropic numbers: $F^- < IO_3^- < BrO_3^- < NO_2^- < ClO_3^- < Br^- < NO_3^- < ClO_4^- < I^- < SCN^-$ (325). In some systems ClO_4^- is stated to occupy a position beyond SCN^- (e.g. 36). Although this series does not follow the order of potencies of I^--transport inhibitors exactly, it merits attention since the more active compounds have the highest lyotropic numbers. Anions that inhibit *p*-amminohippurate concentration in kidney slices also follow this series (302). A large number of effects on colloids such as salting out, swelling, gelation and solvation, viscosity, as well as heats of hydration, etc., of these anions, lead to the same arrangement (75, 76), and Voet (324) has suggested that it is the ionic field strength surrounding the ions that determines their relative positions.

Factors affecting the field strength may also determine "ionic size," and several such correlations, e.g. between heats of hydration and ionic radii, have been made (249, 324). The size factor was suspected on the basis of the comparative behavior of the various halides. This was emphasized recently by Anbar et al. (5, 6), who suggested that the inhibitory properties for iodide uptake by thyroid tissue were functions of their size. They revived an old finding (36) that certain anions of a size similar to ClO_4^-, i.e. BF_4^-, $PO_2F_2^-$, and SO_3F^-, shared certain biological properties of that anion. Like ClO_4^-, these anions inhibited iodide transport; BF_4^- and ClO_4^- were, in addition, shown to be concentrated by thyroid glands (6, 185). The calculated ionic volumes of these anions as well as of ReO_4^- and TcO_4^-, were approximately $4 \cdot 10^{-23}$ cm^3, where I^- is $4.22 \cdot 10^{-23}$ cm^3. They suggested that affinity for thyroid tissue required an ionic volume of this magnitude. However, it was found that the K_m values for these anions were not the same (348). Other size parameters in better agreement with anion saturation properties for thyroid tissue were therefore sought. Measurements by Wolff and Maurey (349) (Table 4) of the inhibitory potencies (K_i) of these anions against thyroidal iodide transport revealed the following series of increasing K_i (or K_m) values: $TcO_4^- \leq ClO_4^- < ReO_4^- < BF_4^- < SeCN^- \cong SO_3F^- < SCN^- < I^- < NO_3^- < NO_2^- < OCN^- \simeq Br^-$. A linear relationship was found to exist between the pK ($-\log K_i$ or K_m) values and the partial molal ionic volumes in the range of 25 to 46 cc/mole (197, 349) (Fig. 4). This suggests that this particular description of ion size may also describe the "binding" site on the hypothetical carrier. It is of interest that the selectivity of certain quaternary ammonium anion-exchange resins can also be correlated with the partial molal volumes of the exchanging anions (115). It thus seems not improbable that an adsorptive or ion-exchange type of process will prove to be important for the specificity of the "active" transport of anions by the thyroid gland.

While the importance of the size parameter appears to be established, other

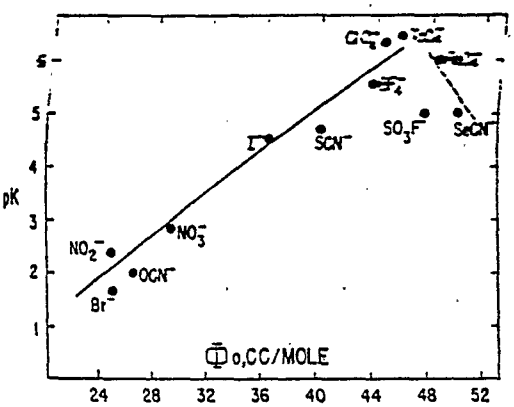


FIG. 4. Relation between anionic size (partial molal ionic volume = Φ_o) and the pK(−log K_m or K_i) for anion transport in sheep thyroid slices. Taken from Wolff and Maurey (349).

factors such as the shape of the anions are also important. The anions known to be concentrated by thyroid tissue are spherical (Br^-, I^-, At^-) or tetrahedral (ClO_4^-, TcO_4^-, ReO_4^-, BF_4^-). On the other hand SCN^-, even though it is a competitive inhibitor (349, 356), is not concentrated in the sense that the other ions are (60, 191, 363, 372, 373) (but see discussion on p. 71). Thiocyanate is linear, but the linear congener $SeCN^-$ is concentrated, albeit poorly. It is to be determined, therefore, whether or not linearity per se is involved or whether other factors, such as metabolic degradation, determine the poor concentration of these anions.

VIII. MECHANISM OF IODIDE PENETRATION

So far we have demonstrated that iodide penetration into the thyroid has the characteristics of active transport, i.e. movement against an electrochemical gradient that depends on the activity or energy change of another system. It appears to be linked to the "ATPase-mediated" transport of K^+ and/or Na^+, but a choice between this and a directly mediated active anion transport serum is impossible at present. In the frog stomach (150a), rat ileum (64a), the skin of the South American frog (384), and the adrenaline-stimulated skin glands of the European frog (169a) transport of Cl^- ion supports the transmembrane potential difference (i.e. contributes to the short-circuit current). While SCN^- appears to depress Cl^- (and H^+) secretion by the stomach (129, and many others since), other anions have not been tried; thus, whether these active processes resemble iodide transport cannot be stated at present. In any case, the iodide fluxes usually observed in the thyroid gland (see Table 3) would not be sufficient to make an easily detectable contribution to the short-circuit current. Most additional work on the transport mechanism for I^- has gone on the assumption that active I^- transport is mediated by a carrier which supplies specificity by some adsorption process. The evidence for this is scant and depends largely on the fact that I^- transport kinetics obey models based on carriers. A "pore theory," with pores containing fixed positive charges whose intensity could be varied by metabolic changes, and which could thus be sensitive to the different field strengths of anions of various "sizes," could probably be constructed that would fulfill most or all the

experimental findings to date (e.g. 225). This has not as yet been done. In this section the chemical evidence for carriers and other transport mechanisms is examined.

It should, perhaps, be re-emphasized that *carrier*-mediated transport does not, of itself, necessarily imply *active* transport against an electrochemical gradient.

A. Chemical Conversion

In a sense the fact that the thyroid converts most of its iodine to organic forms and then later releases part of this again to iodide ion can be construed as an iodide-concentrating mechanism. However, it seems unjustified to class this as a transport mechanism in the usual sense. The only case in which such a mechanism might be operative for the purpose of transport is in certain algae. The iodide-concentrating mechanism of the brown algae can be divided into two classes: those that can operate in the presence of reducing agents (like antithyroid compounds), and those that cannot. Into the first class we can place the algae *Fucus ceranoides* and *Fucus vesiculosus* (168, 337), which behave in this respect like the thyroid gland. Into the second class fall several members of the genus *Laminaria* (257, 276), *Nereocystis*, and probably *Nitella* (307). The iodide-concentrating *Ascophyllum* has not yet been classified (165). In algae of this class oxidation of iodide appears to be a prerequisite for uptake, since the process is inhibited by antithyroid agents, etc. (257, 276, 307). Since *Laminaria* readily oxidizes iodide to I_2 or related forms (65, 173, 257, 276) yet contains ample quantities of iodide ion (173, 258, 276), Shaw (276) has proposed that iodine enters the tissues in an unionized form (HIO) and is then reduced again. Present evidence suggests that this is not the mechanism by which mammalian tissues, such as the thyroid, salivary, or mammary glands, concentrate iodide because maximum $T/M[I^-]$ values are attained in the presence of antithyroid agents that prevent the oxidation of the halide. Furthermore, fully oxidized anions such as ClO_4^-, TcO_4^-, and ReO_4^- are concentrated, a finding that effectively rules out the need for oxidation as a transport step (6, 185, 348).

B. Carriers

An iodide carrier might be expected to contain one or more positively charged groups that could be relatively easily discharged by substitution of another anion or by a small pH change, etc. An onium group of N, S, or conceivably P, would be groups to consider. On the basis of the sensitivity of anion transport to the partial molal anionic volumes (349), the carrier may exhibit similarities to ion exchangers. If there is a carrier it must, in addition, function in concert with normal organization of the cell since intact cell structure is required for iodide transport (see section III). Whether this involves a polarization of the carrier or other factors can only be conjectured.

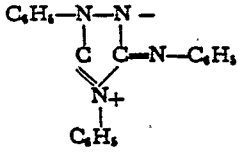
1. Proteins as iodide carriers. The only biological compounds so far considered for carrier-roles are proteins and phospholipids. It has been known for some time that serum albumin has a moderate affinity for iodide (and SCN^-) (54, 267). There are two sites for SCN^- with affinity constants of 1000 and 25 (268). Constants for

iodide are believed to be similar. The order of increasing affinities for serum albumin is $Cl^- < Br^- < NO_3^- < I^- \simeq SCN^-$. Perchlorate behaves like SCN^-. As far as studied, this series describes an increasing order of partial molal ionic volumes, etc., and thus is a parallel of the potency of these anions in inhibiting iodide transport in the thyroid (349) (see also p. 76). This therefore suggests that similar proteins might be involved in this process. No very strong I^- association has been found in dilute or concentrated homogenates of thyroid tissue when adequate precautions have been taken that no organic iodine is allowed to form (72, 348, 377), although some association of TcO_4^- and ReO_4^- has been shown (348). In equilibrium dialysis experiments purified thyroglobulin has a low affinity for iodide (349) and TcO_4^- or ReO_4^- (Wolff, unpublished observations). Furthermore, certain nonthyroglobulin amonium sulfate fractions of beef thyroid tissue showed a low order of affinity for I^- (Wolff, unpublished observations). Nevertheless, a certain amount of anion association may occur, and it should be kept in mind that the affinity of serum albumin for iodide ion may lower the availability of iodide for transport into the thyroid gland (356).

An extraordinary type of iodide binding, probably to protein, is found in the plasma of migratory teleosts (genus *Salmo* and *Alosa*). Leloup and Fontaine (see 180) have found that iodide ion in sera from salmon, trout, shad, etc., is bound to "albumin" to such an extent that it lowers iodide penetration into red cells, is not dissociated from the protein during paper electrophoresis, and gives ratios of 10 to 20 in favor of the protein compartment in equilibrium dialysis. We have confirmed the latter in plasma of *Salmo gardnerii*, which yielded ratios of 20 to 40 at 10^{-6}M I^- (Wolff, unpublished). Anion specificity has not yet been determined. The intensity of iodide binding is related to the migratory pattern and may serve as an additional means for retaining this element in an environment in which it is scarce. Most other sera give ratios near 1.0.

2. Phospholipids as iodide carriers. Phospholipids, and especially lecithin with a quaternary nitrogen[6], are obvious choices for complexing anions and making them lipid-soluble. It has been known for years that a small fraction of serum Cl^- and SCN^- is extractable into nonpolar solvents (58, 258a), and lecithin has been credited with causing this effect. Vilkki (320, 321) has found that lecithin fractions from several organs will promote iodide solubility in chloroform. A thyroidal leci-

[6] It has long been known that nitron



forms insoluble salts with many anions, especially the monovalent tetrahedral species which are also the most potent inhibitors of iodide transport (330). The nitron-anion complexes are soluble in nonpolar solvents, and Schneider and Wolff have shown that solubility of anions such as I^-, SCN^-, ReO_4^-, and TcO_4^- in chloroform is increased > 100-fold in the presence of an excess of nitron (unpublished). Quantitative interpretation of such distributions is complicated by lack of knowledge of distribution coefficients of *all* components.

thin fraction appears to be much more potent in this respect than lecithin from any other source so far tried. We have attempted to evaluate the significance of such anion binding by comparison of the affinity of the phospholipid for the anions with the K_m or K_i of sheep thyroid slices for various anions (349) (see p. 76). The distribution coefficients in heptane containing thyroidal "lecithin" give the general order $TcO_4^- > ReO_4^- \geq SCN^- > I^- > Br^- \gg SO_4^=$, $WO_4^=$ or $MoO_4^=$ (272). These anions also diminish I^- solubility in such "lecithin"-containing solvents in the same sequence of potencies. While this is the same order seen in thyroid slices, the spread of values of distribution coefficients is much smaller than that of the K_m or K_i values. The sequence is, furthermore, a function of the phospholipid concentration. This suggests a rather more complicated process.

The requirement for K^+ ion and the inhibition by cardiac glycosides of iodide transport led to an investigation of these factors in phospholipid synthesis by thyroid slices (322). This process is influenced by K^+ and cardiac glycosides in the same manner as is iodide transport. Whether K^+ and cardiac glycosides influence phospholipid synthesis and hence transport, or whether they influence transport and hence phospholipid synthesis, remains to be established. At present, the role of lecithin in iodide transport should be considered primarily as a promising area for research.

IX. FACTORS THAT INFLUENCE IODIDE TRANSPORT

A. Thyrotropin

Thyrotropin is by far the most important controlling factor for iodide transport. So far, no convincing evidence has been obtained of an effect on the T/M[I^-] of TSH added to thyroid tissue in vitro. Much of the evidence obtained for TSH effects has been inferred from manipulations of the diet, treatment with antithyroid drugs, etc. The evidence has been reviewed in some detail by Halmi (125), but a summary of the findings will be presented here. The important conclusion is that the effect of TSH is *indirect*, apparently secondary to other changes in the gland.

1. Exogenous TSH. Exogenous TSH leads to an increase in the T/S[I^-] of various animals (122–124, 133, 134, 274, 297, 303, 317, 318, 345). The latency for this stimulation is longer than that for most other responses of the thyroid gland to TSH, e.g. histological signs of activation, phospholipid synthesis, stimulation of glucose oxidation, or release of iodine from the gland (82, 89, 134, 212, 318, 336). In rats the time required to see a significant effect on the T/S[I^-] is 6 to 8 hours. The T/S[I^-] reaches a maximum at 24 to 48 hours after a single dose of TSH, and the stimulation is dissipated in another ~48 hours. A biphasic response, with an initial drop in the T/S[I^-] followed by a rise, has been reported in the rat (127). The nature of this depression is not understood but has been explained by an early increase in the exit rate constant (360) or the stimulation of proteolysis of thyroglobulin, followed by deiodination of iodotyrosines and subsequent release of I^- (2, 214, 215, 259, 260, 261). Alternatively, an inhibitory organic iodine compound could be formed. No satisfactory explanation has yet been produced. Iodide release occurs also in the gland blocked with antithyroid agents, suggesting the possibility that the unlabeled iodide liberated by deiodination can communi-

cate with the exchangeable iodide pool and lead to discharge of labeled iodide. Direct proof for this has not yet been supplied. Epinephrine and norepinephrine also cause iodide release (2).

The mechanism of stimulation of iodide transport by TSH may well reside in the stimulation of the Na^+-K^+-activated ATPase activity (345). This shows marked stimulation on TSH treatment whereas the nonspecific ATPase activity shows only a slight increase (Fig. 3). The important finding that this stimulation occurs in vitro, and very rapidly (312), suggests the possibility that a number of the other early responses to TSH may be secondary to an effect on this ATPase activity, but raises the problem as to why a TSH effect on the T/S[I^-] is so slow to appear. The early stimulation of CO_2 production from glucose-6-^{14}C produced in vitro by TSH (82) is not inhibited by ouabain (Pastan and Wolff, unpublished data).

2. *Hypophysectomy.* Hypophysectomy reduces the T/S to low values (37, 38, 112–114, 122, 123, 242, 303, 306, 317). The level attained appears to be a result of many variables including the preoperative T/S[I^-], diet, time after hypophysectomy, and postoperative dietary history. Values may get as low as 1 to 2 in animals on high iodine intakes and tested sufficiently long after hypophysectomy. On the other hand, mice on lower iodine intake may show only a moderate depression in the T/S[I^-]. The depression can be corrected by TSH to normal or greater levels in the absence of histological signs of thyroid activation. The major effect of hypophysectomy appears to be on the unidirectional iodide clearance by the gland (110, 111, 360, 362), but there is also a decrease in the K_{TB} (see Table 3).

Other manipulations of endogenous TSH have the expected effect on the T/S[I^-]. These include: *a*) treatment with goitrogens (132, 274, 314, 316, 360, etc.); *b*) low-iodine diets (123, 125, 284, 369, 371); *c*) treatment with T_3 or T_4 (e.g. 111, 126, 136, 274, 318, 381); *d*) treatment with agents that displace T_4 from circulating binding proteins (59, 222, 250, 353); *e*) elevated ambient temperature (105). The first two most probably raise the level of TSH and with it the T/S[I^-]. Kinetic analysis has revealed that the effect is primarily on the unidirectional clearance of iodide by the thyroid (131, 360, 362). If a goiter develops there may be a depression in T/S[I^-] (124, 136, 186, 274). Part is due also to an increase in the exit rate constant, K_{TB} (124, 360, 362).

The other manipulations of the TSH level mentioned above—treatment with thyroid hormones, liberation of bound thyroxin, or elevated ambient temperatures—all lower the T/S of I^- and presumably the T/S of other anions.

The T/S for various other anions is also increased by TSH or conditions that tend to increase endogenous TSH output. These anions include T/S[At^-] (73, 278, 279), T/S[ReO_4^-] (277), T/S(ClO_4^-], and T/S(BF_4^-] (185). In the case of SCN^- there appears to be a stimulation of its breakdown (266).

In addition it can be shown that the hypophysectomized animal without TSH or on a constant supply of it can still respond to dietary manipulations with an increase in the T/S[I^-] (110, 123, 131, 133, 317, 360) and other parameters of thyroid function (56, 104, 335). The major effect is again on the clearance of iodide with only small effects on the K_{TB} (two-compartment model) (110, 131).

B. Thyroid Iodine Content

Thyrotropic stimulation lowers the total (and especially the hydrolyzable) iodine of the thyroid (13, 194, 338). Other means of lowering thyroidal iodine are low-iodine diets or treatment with an antithyroid agent (123, 284, 317, 360, 362). All these procedures increase T/S[I⁻]. While these factors undoubtedly operate via the pituitary-thyroid servomechanism in the intact animal, they can be shown to act independently as well, and it has been postulated that some organic iodine component of the thyroid also controls iodide transport (123, 317). However, TSH stimulation and thyroid iodine content stand in such relation to each other that it is difficult in many experiments to tell which is the cause of effects on T/S[I⁻]. The fact that the latent period for release of iodine is shorter than that for an effect on T/S (134, 336) supports the possibility of an indirect effect. In iodine-depleted mice with high T/S[I⁻] it takes surprisingly little organic iodine formation to depress T/S[I⁻]. There was little effect of organic iodine formation on the T/S of iodine-rich glands (359). It seems possible that newly formed organic iodine is a better inhibitor of T/S[I⁻] than organic iodine of an older vintage.

The nature of the inhibitory organic iodine compound has not been established. Ample substantiation of a depressing effect of injected thyroid hormones has been published (126, 134, 136, 157). Much of this effect results from inhibition of endogenous TSH secretion, but since T/S[I⁻] values are lower in the presence of excess thyroid hormone than after hypophysectomy, it has been suggested that there is an additional depressive effect (111, 126). Its nature is obscure but appears to involve an increase in the exit rate constant (111). It has also been claimed that certain thyroid fractions depress the T/M[I⁻] of slices (205), but the various iodinated amino acids seem not to be inhibitory under such conditions (124, 136, 352). Recently, several iodinated peptides have been isolated from thyroid tissue that can inhibit organic iodine formation in vitro (187). It was not stated whether this was inhibition of iodide transport or organification.

C. Other Factors

1. Species or strains. Species or strains can be compared only roughly since other variables, especially diet, have not generally been controlled. It is apparent from Table 3 that despite identical diets rats and mice show remarkable differences of T/S[I⁻]. The difference between the high ratios of mice and the lower ratios in rats are, to a large extent, functions of the lower exit rate constants of mice. On the other hand, these differences may be due to the particular strains of rats and mice used. Certainly, among various strains of mice there may be wide variations in the T/S[I⁻] found at 1 hour after $^{131}I^-$ administration (281–284). Whether strain and species differences reside primarily in the thyroid, pituitary gland, or elsewhere is not certain at present.

2. Sex. In most strains of mice and rats the thyroid of the male establishes a higher T/S[I⁻] than the female (281–283). In female rats there are fluctuations with the estrous cycle with lowest values during proestrus and a peak in late estrus (35). In the mouse lowest T/S[I⁻] values are found in diestrus with a maximum in proestrus (34). At present, there is disagreement regarding the effect of estradiol (39, 81). Part of this effect may be mediated by the pituitary gland (118).

3. Age. Changes in age of experimental animals show some correlation with certain parameters in thyroid function (180, 227, 284). It has been suggested that the increase in T/S[I^-] with age is a result of maturation in pituitary TSH secretion (182).

4. Blood flow. While no measurements have been made on the effect of blood flow on T/S[I^-], there is ample evidence that vasoconstriction severely limits $^{131}I^-$ uptake by the thyroid gland (16, 48, 196, 288). Divergent results have been reported with the sympathomimetic amines given either directly or increased reflexly, but whenever vasoconstriction has been observed $^{131}I^-$ uptake has been low. When the circulation is completely stopped there is a rapid fall of thyroid I^- (364); it is, however, not easy to predict the effect of other changes in blood flow on T/S [I^-].

X. IS THE ABILITY TO CONCENTRATE IODIDE NECESSARY?

The thyroid gland may be described as an efficient collector of a rare element, iodine; a machine for the synthesis of the thyroid hormones; and a commodious storehouse for the finished product. We may now ask whether the first step is a prerequisite for the other two. It has, of course, been known for some time that organic iodine formation does not require intact cells or active iodide transport. (Whether these in vitro pathways are similar to those of the intact thyroid is by no means certain, however.) The evidence for intact cells comes from studies on pathological thyroid tissue. Wollman et al. (369) have described transplantable mouse thyroid tumors that could accomplish no more than a T/S of ~0.4 while still able to make small amounts of organic iodine. Furthermore, two patients with goiter and hypothyroidism have now been described in whom the ability to concentrate iodide was lost in the thyroid [as well as in gastric mucosa and salivary gland (294, 351)]. On the assumption that the remainder of the biosynthetic apparatus was intact, both patients were treated with large doses of iodide alone. Both responded well (growth, BMR, cholesterol, etc.). This suggests strongly that under especially favorable conditions enough I^- can be made to enter the thyroid by "diffusion" to allow production of normal amounts of the thyroid hormone. These favorable conditions are almost universally absent, and were it not for the "active" transport of iodide normal thyroid function could not be attained.

XI. SUMMARY

The thyroid gland possesses a mechanism for accumulating iodide ion in concentrations ten to several hundred-fold greater than that of the surrounding medium. The high values are attained only in glands in which organic iodine formation is blocked by an antithyroid agent of the thiouracil or sulfonamide type, but significant concentration gradients are also attained in glands free to form organic iodine. The ability to concentrate I^- is shared by a number of other organs—gut, stomach, salivary glands, choroid plexus, ciliary body, and skin. The concentration process occurs in vivo and in vitro, does not require organization into follicles, but does require cellular integrity. The concentration gradient, T/S[I^-] or T/M [I^-], is established against both an electrical and a chemical potential and has various other characteristics of active transport such as a requirement for aerobic

metabolism and ATP production, inhibition at low temperatures, and competitive inhibition by related anions. Compared to cation transport, there is a rather surprising lack of specificity for the anions, which not only inhibit I^- transport, but also discharge accumulated I^- and, as far as tested, are concentrated by the thyroid (with the exception of SCN^-). Including among these univalent anions are, in addition to I^-: Br^-, OCN^-, NO_2^-, NO_3^-, At^-, SCN^-, SO_3F^-, $SeCN^-$, BF_4^-, ReO_4^-, ClO_4^-, and TcO_4^-. From simple kinetic models, constants can be obtained that permit these anions to be related to each other as functions of their partial molal ionic volumes (i.e. size), peak values in K_m or K_i occurring with TcO_4^- and ClO_4^- at volumes of ~46 cc/mole.

The ability to transport K^+ and Na^+ must remain intact for iodide transport to occur, and ouabain, an inhibitor of cation transport, depresses T/M[I^-]. The ouabain inhibition appears to act through a Na^+-K^+-requiring ATPase activity of thyroid tissue which is linked in an unknown manner to iodide transport. Very preliminary evidence for an iodide carrier has been offered, but whether or not the ATPase activity involves formation and breakdown of such a carrier is a matter of speculation.

Steps may well exist in addition to those supplying energy for and specificity to the iodide-concentrating process, but they have yet to be defined.

The main control mechanism that adjusts the rates of and capacity for iodide transport is the level of thyrotropic stimulation. This may be influenced, in turn, by diet and antithyroid agents or other drugs. In addition, the intrathyroidal level of some organic iodine compound, as well as age, species, and circulatory adjustments, play a role in the control of iodide transport.

This review owes much to frequent stimulating discussions with Dr. S. H. Wollman of the National Cancer Institute. I am also indebted to Drs. G. Andros, N. S. Halmi, and D. M. Woodbury for providing manuscripts before publication.

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